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INVESTIGACIÓN DE LA ASOCIACIÓN ENTRE MANIFESTACIONES CUTÁNEAS E INFECCIÓN POR PARVOVIRUS B19

TESIS DOCTORAL

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A mi padre.

Introducción General

a) *Presentación de los trabajos*

Los trabajos compendiados que constituyen el cuerpo de la presente tesis están relacionados con la infección por el Parvovirus humano B19 (B19V), los cuadros dermatopatológicos ocasionados por la misma y la hipótesis de que la detección aislada en biopsias cutáneas de ácido desoxirribonucleico (ADN) de B19V no constituye necesariamente prueba de relación causal entre aquella y estos, pues el virus es capaz de permanecer en los tejidos mucho tiempo después de ocurrida la infección. Tal relación debe basarse en determinaciones analíticas (seroconversión, presencia de ADN vírico en sangre) o confirmación de la presencia de material del virión en los tejidos, como la demostración de proteína vírica (Viral Protein 2, VP2) en el endotelio de vasos dérmicos. Esta última apoya la tesis de que el virus penetra en células generalmente poco permisivas valiéndose de un mecanismo de estímulo mediado por anticuerpos, en el curso de la primera fase de la infección.

En el primero de los trabajos (***Immunohistochemistry in the Diagnosis of Cutaneous Viral Infections***) se detalla la aportación de la inmunohistoquímica al diagnóstico de infección reciente por B19V, en el marco de un estudio global de infecciones víricas de la piel. En el segundo (***Detection of human parvovirus B19 DNA in 22% of 1,815 cutaneous biopsies of a wide variety of dermatologic conditions suggests viral persistence after primary infection and casts doubts on its pathogenic significance***) se estudian los patrones histopatológicos de una amplia serie de muestras

de biopsia con positividad para ADN de B19V, planteándose la falta de correlación entre esta circunstancia y la patogenicidad del virus; y en el tercero (***Immunohistochemical demonstration of Parvovirus B19 VP2 protein in periflexular exantema in an adult, supporting antibody-dependent enhancement as means of endotelial uptake of the virus***) se amplia la detección inmunohistoquímica en biopsia de proteína de B19V a un ejemplo de erupción flexural como manifestación de primoinfección.

b) *Justificación de la temática*

El B19V, el mejor conocido de los parvovirus capaces de infectar al ser humano (1-3), es el agente causal de la enfermedad exantemática infantil denominada "quinta enfermedad" (eritema infeccioso, EI) (8). Está involucrado asimismo en cuadros reumatológicos (artropatía aguda o crónica (9)), aplasia medular transitoria y anemia en pacientes inmunodeprimidos, y contribuye a la patogénesis de una de las formas de hidrops fetal de origen no inmunológico (9). Además, se ha detectado ADN o VP2 de B19V por diferentes métodos en numerosos tejidos (médula ósea, cerebro, intestinos delgado y grueso, corazón, riñón, hígado, pulmón, tejido linfoide, estómago, sinovial, testículo y tiroides). Por este motivo, se ha implicado a B19V en la patogénesis de una amplia gama de enfermedades, que comprenden la miocardiopatía dilatada, la enfermedad de Kikuchi-Fujimoto, linfomas, gangrena no oclusiva de estómago e intestino, carcinoma de intestino grueso, tumores testiculares y enfermedades tiroideas(10).

Se han descrito tres genotipos de B19V, que se diferencian en un 10% de su genoma (3,11): el genotipo 1 (el más extendido), el genotipo 2 (restringido a sujetos nacidos antes de 1973), y el genotipo 3 (detectado en África occidental y escasos países occidentales) (12). El virus se identificó en 1975 en el suero de nueve donantes de sangre sanos, un paciente con hepatitis aguda y otro portador de un trasplante renal reciente, en forma de resultados falsamente positivos en el cribado rutinario de suero para detectar antígeno de superficie de la hepatitis B (13). Desde que en 1983 se confirmara su relación con la quinta enfermedad (8) (a su vez conocida desde el siglo XVIII(14)), se ha descrito su asociación con diversos procesos dermatológicos, implicándolo en la patogénesis de numerosos cuadros clínicos. Estos abarcan desde entidades infrecuentes con una fuerte asociación etiológica con B19V, como la erupción papular y purpúrica "en guantes y calcetines" (EPPGC) (15), hasta afecciones dermatológicas clásicas sin aparente relación con el virus. La lista de entidades incluye la púrpura de Henoch-Schönlein, el eritema multiforme, erupciones semejantes a dermatomiositis, urticaria crónica, esclerosis sistémica, pitiriasis liquenoide, psoriasis, enfermedad de Behçet, granuloma anular, síndrome de Wells, lupus eritematoso y esclerodermia (4-6,16-43)

La asociación causal entre B19V y las diferentes enfermedades (cutáneas y sistémicas) con las que se lo ha relacionado se ha basado en determinaciones analíticas de diferentes tipos: serología para detección de anticuerpos (IgM seguido de seroconversión), detección de ADN de B19V en suero o tejido, y detección de proteínas víricas (VP2) en tejido mediante inmunohistoquímica (10). Sin embargo, la detección del ADN de B19V mediante reacción en cadena de la polimerasa (PCR) en piel sana y en lesiones cutáneas muy dispares sugiere que B19V persiste en los tejidos mucho tiempo

después de la infección inicial (para esta persistencia se ha creado el término “Portafolios biológico” (44)) , lo que restaría valor a la mera detección del ADN. Los trabajos previos a los aquí presentados han estudiado casos aislados o series cortas de pacientes. Así, se encontró ADN de B19V en biopsias cutáneas de 9 pacientes con pitiriasis liquenoide (32); en 18 de 36 pacientes con urticaria crónica y 14 de 22 sujetos sanos (30); y en 30 de 121 con piel normal, nevus, melanomas o pitiriasis liquenoide.(45)

Por otro lado, la demostración en biopsias de piel mediante inmunofluorescencia o inmunohistoquímica de la proteína vírica VP2 se ha comunicado en muy escasas ocasiones en pacientes con cuadros clínicos asociados (por estudio serológico) a infección por B19V. Así, Schwarz et al (17) detectaron mediante inmunofluorescencia indirecta positividad en los queratinocitos epidérmicos en la muestra cutánea de un eritema infeccioso en un niño. Takahashi et al (46) observaron mediante inmunohistoquímica material positivo granular en el citoplasma de las células endoteliales de la dermis en una paciente de 37 años con una erupción generalizada, y partículas víricas en la misma localización mediante microscopia electrónica. Tanto los queratinocitos como el epitelio de glándulas ecrinas y el endotelio de vasos dérmicos mostraron positividad inmunohistoquímica para la proteína VP2 en tres pacientes con EPPGC en el trabajo de Aractingi et al (5). En aportación más reciente se observó por nuestro grupo positividad mediante inmunohistoquímica en el citoplasma de células endoteliales dérmicas en tres casos de EPPGC (7).

c) *Aportación original*

He estudiado mediante PCR la presencia de ADN de B19V en un gran número de biopsias dermatológicas, caracterizado los diferentes cuadros histopatológicos y entidades clínicas correspondientes, y contribuido con ello a una mayor precisión en la asignación del virus a la patogenia de las numerosas enfermedades cutáneas con las que se lo ha relacionado en la literatura previa. Además, he aportado datos inmunohistoquímicos que certifican la presencia de B19V en biopsias cutáneas, en diferentes exantemas en el curso de primoinfección (erupción purpúrica y papular "en guantes y calcetines" y exantema flexural).

Resumen de los resultados obtenidos

En el estudio molecular realizado (PCR) se detectó ADN de B19V en 402 de 1.825 biopsias cutáneas (22%), correspondientes a 1.749 pacientes.

Los cuadros dermatológicos que motivaron la toma de biopsia en los 402 casos positivos se clasificaron en once grupos:

1) Patología inflamatoria de la unidad folículo-sebácea-apocrina

2) Dermatosis papulosas, eritematosas, descamativas y urticariformes

3) Enfermedades ampollosas

4) Dermatosis anulares-policlíclicas

5) Enfermedades granulomatosas, histiocitosis, mastocitosis

6) Enfermedades infecciosas

7) Lesiones nodulares

8) Vasculitis y procesos de reactividad vascular

9) Depósitos

10) Colagenosis

11) Malformaciones, hamartomas, hiperplasias y neoplasias benignas

En estos once grupos se recogen un total de 586 entidades clínicas o clinicopatológicas, pues en muchas ocasiones se aportaba más de una opción diagnóstica por el dermatólogo que remitía el caso.

Las entidades más frecuentes fueron vasculitis (40 casos), pitiriasis liquenoide (38), pitiriasis rosada (33), dermatitis-eczema (27), liquen plano-liquen nitidus (19), psoriasis (18), púrpura (18), eritema crónico migratorio-borreliosis (17) y granuloma anular (16).

Los patrones histopatólógicos de dichas muestras abarcaron todo el espectro de afectación histológica (perivascular y variantes, nodular, difuso, vasculitis…) reconocidos en la literatura (47)

Por otro lado, la detección inmunohistoquímica de VP2 fue únicamente observada en tres casos de SPPGC y en una erupción mixta flexural y purpúrica (esta última, objeto de una publicación independiente).

Estos resultados apoyan la persistencia de B19V en el organismo humano tras la primoinfección (en la infancia o en la edad adulta), aunque se desconoce el mecanismo exacto de latencia-persistencia vírica. Solamente si se detecta ADN tisular en el contexto de una infección aguda demostrable serológicamente (conversión de anticuerpos, detección de ADN de B19V en suero), puede atribuirse el cuadro clínico al virus. La detección de VP2 en el interior de células implica participación patogénica del virus, quizá con captación estimulada por mecanismo mediado por anticuerpos.

En conclusión, los datos aportados (positividad para ADN vírico mediante PCR en el 22% de las muestras) indican que es posible detectar ADN de B19V en gran número de procesos dermatológicos, que abarcan numerosas entidades clínicas y todo el espectro de patrones lesionales histopatológicos. Por lo tanto, el simple hallazgo de B19V en muestras de tejido no basta para probar una relación causal con la enfermedad en cuestión. Estos datos presumiblemente puedan validarse en muestras de otros órganos (corazón, tiroides…), en los que se ha asumido igualmente una participación patogénica de B19V sobre la base de resultados moleculares.

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Immunohistochemistry in the diagnosis of cutaneous viral infections

Part I.
Cutaneous viral infections by herpesviruses and papillomaviruses.

American Journal of Dermatopathology. 2015 Volumen 37. Número 1. Páginas:1-14.

Part II
Cutaneous viral infections by parvoviruses, poxviruses, paramyxoviridae, picornaviridae, retroviruses and filoviruses.

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Immunohistochemistry in the Diagnosis of Cutaneous Viral Infections—Part I. Cutaneous Viral Infections by Herpesviruses and Papillomaviruses

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Background: Cutaneous viral infections are of increasing clinical importance, particularly in patients who are immunocompromised.

Objective: The use of immunohistochemistry (IHC) in the diagnosis of cutaneous infections provides a rapid morphological diagnosis and can be applied to confirm the diagnosis of specific viral infections that may be difficult to diagnose with certainty using routine microscopy alone, thus facilitating clinical decisions in patient care.

Methods: Several immunostains for specific viruses that have been useful in dermatopathology are reviewed. Emphasis is placed on new stains and novel uses of existing stains.

Results: This article is an up-to-date overview of the potential uses and limitations of IHC in the histopathologic diagnosis of cutaneous viral infections by herpesviruses and papillomaviruses.

Limitations: Whereas specific monoclonal antibodies effectively distinguish infections by herpes simplex virus-1, herpes simplex virus-2, varicella zoster virus, Epstein–Barr virus, and cytomegalovirus, IHC does not distinguish between the 120 antigenically distinct strains of human papillomavirus.

Conclusions: IHC may assist dermatopathologists to appropriately diagnose viral infections caused by herpesviruses and papillomaviruses.

Key Words: immunohistochemistry, virus, skin, infection, herpesviruses, papillomaviruses

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LEARNING OBJECTIVES

Upon completion of this learning activity, participants should be better able to:

1. Use immunohistochemistry (IHC) to identify viral pathogens that are relevant to dermatopathology.
2. Apply these techniques in the diagnosis of cutaneous viral infections and other related diseases.

INTRODUCTION

Various laboratory techniques can be used to assist in the specific diagnosis of a suspected viral disease. These include light and electron microscopy of a biopsy or smear, serology, viral culture, and immunomorphological methods. Although viral isolation in tissue culture remains the paramount diagnostic method, the development of monoclonal antibodies to various viruses, for use with fluorescent, immunoperoxidase, and enzyme-linked immunosorbent assay techniques, has made possible the rapid diagnosis of many viral infections with a high degree of specificity.[1] These markers cannot replace the routine histologic study; however, they can help to narrow the differential diagnosis.

Immunohistochemistry (IHC) continues to be one of the main adjunctive methods to conventionally stained sections in histopathology. This is mainly related to the fact that it is a relatively simple, fast, and inexpensive method.[2] Because of technical advances, there has been a significant increase in the number of diagnostic immunohistochemical stains available for pathologists and dermatopathologists in recent years. The sensitivity and specificity of certain antibodies, their pattern of staining (nuclear, cytoplasmic, or membranous), and background artifact must be considered in their interpretation. In addition, evaluation must be done in relation to internal control.[3] IHC, however, is only a tool to be used appropriately in the context of clinical and histopathologic correlation; unreasonable use can be misleading and financially cost ineffective.[4]

In this review, we highlight the main immunohistochemical available techniques that have been used and continue to evolve in the diagnosis of mucocutaneous viral infections and discuss their applicability.

IMMUNOHISTOCHEMISTRY

IHC has become an increasingly important histopathologic tool over the past 20 years, and is now a key part of

routine practice of dermatopathologists. IHC begins with antigen retrieval by pretreating the tissue to unmask antigens hidden by fixatives, such as formalin. This step is often done by microwaving the tissue in citrate buffer or other agents, such as pepsin, proteases, or trypsin. Primary antibody is then applied, which binds the antigens of interest. After washing off the excess primary antibody, a secondary antibody is added to bind the primary antibody. This is then followed by the addition of an enzyme complex, such as avidin–biotin–peroxidase complex, and a chromogen.[2]

Immunoperoxidase techniques involve the use of enzyme labels that convert a colorless substrate into a colored one. The most widely used enzymes are peroxidase and alkaline phosphatase, and the most widely used substrates are diaminobenzidine, amino ethylcarbazole, and nitroblue tetrazolium, which, respectively, produce brown, red, and blue colors. These markers can be attached, or conjugated, directly to the primary antibody or indirectly using secondary antibodies or substances, such as biotin and protein A.[5]

The use of a continuously increasing number of newly raised and commercially available antibodies for IHC has tremendously broadened IHC applicability, this being especially relevant for the diagnosis of different types of infections.[2,4,6] The use of IHC in the diagnosis of cutaneous infections has recently been applied to confirm the diagnosis of specific viral infections that may be difficult to diagnose with certainty using routine microscopy alone.[2,4,7,8] Table 1 lists the main commercially available antibodies for specific viruses that can be useful in dermatopathology.

TABLE 1. Available Antibodies for Specific Viruses Useful in Dermatopathology

Antibody Against	Type/Clone
HSV-1	Rabbit polyclonal
	DAKO
HSV-2	Rabbit polyclonal
	DAKO
VZV	Monoclonal, mouse
	C90.2.8
	Menarini
EBV	Monoclonal, mouse
	CS1-4
	DAKO
CMV	Monoclonal, mouse
	CCH2 + DDG9
	DAKO
HHV-6	Monoclonal/OHV-1, OHV-2, p41
HHV-7	Monoclonal/HHV-7 pp85
HHV-8	Monoclonal, mouse
	13B10
	Menarini
Papillomaviruses (HPV)	HPV L1: monoclonal, mouse
	Cytoimmune diagnostics
	HPV cocktail: monoclonal, mouse
	BPV-1/1H8 + CA
	Zytomed systems
Parvovirus B19 (PVB19)	Monoclonal, mouse
	R92F6
	Menarini
Merkel cell polyomavirus (MCPyV)	Monoclonal, mouse
	CM2B4
	Santa Cruz Biotechnology
SV40 [trichodysplasia spinulosa polyomavirus (TSPyV)]	Monoclonal, mouse
	Pab101
	BD Biosciences
HTLV-1	Monoclonal, mouse
	1A3
	Biozol
Measles	Polyclonal, rabbit
	Acris
Enterovirus	Monoclonal, mouse
	DAKO
ORF *Parapoxvirus* (ORF)	Monoclonal
Ebola virus (EBO)	EBO antigen

VIRAL PATHOGENS

Viruses are separated into families on the basis of the type and form of the nucleic acid genome, of the morphological features of the virus particle, and of the mode of replication. There are 4 important families involved in cutaneous viral diseases: the DNA families of Herpesviridae, Papillomaviridae, and Poxviridae, and the RNA family Picornaviridae.[1] In addition to these 4 families, exanthemas can occur in the course of infections with the following families: Adenoviridae, Reoviridae, Togaviridae, Flaviviridae, Retroviridae, Parvoviridae, Paramyxoviridae, Arenaviridae, Filoviridae, and Bunyaviridae.[2,4,7,8] The 3 major DNA families produce lesions that are histologically diagnostic for a disease or group of diseases, whereas the other viruses, particularly the RNA viruses, produce lesions that are often histopathologically nonspecific, usually showing epidermal spongiosis and superficial perivascular lymphohistiocytic infiltrate.

MATERIALS AND METHODS

The biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. For routine histology, 5-μm-thick sections were stained with hematoxylin and eosin. Immunohistochemical staining was performed both at the Pathology Department of Fundación Jiménez Díaz University Hospital in Madrid and the Dermatopathology Laboratory at Friedrichshafen, Germany. Tissue sections were processed on a BioTek Solutions TechMate 500 (Dako) where 1-hour incubation with a primary antibody was performed. The immunohistochemical study of the viral pathogens was performed using the commercially available antibodies described in Table 1. Several antibodies for specific viruses described in the literature were not commercially available at the time of elaboration of the article; therefore, immunohistochemical staining for these pathogens could not be tested.

HERPESVIRUSES

More than 80 herpesviruses have been identified, 8 of which are known human pathogens. Herpes simplex viruses

(HSVs) belong to the ubiquitous Herpesviridae family of viruses, which comprises HSV-1, HSV-2, varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), human herpesviruses (HHV) 6 and 7, and Kaposi sarcoma (KS)–associated HHV-8.

Herpes Simplex Virus and Varicella Zoster Virus

Histopathologic findings of biopsies of fully developed labial and genital vesicles because of HSV and vesicles caused by VZV consist of an intraepidermal blister with varying degrees of epithelial necrosis.[9] Two mechanisms are involved in the formation of intraepidermal vesicles: one is the ballooning of keratinocytes and the other is the reticular degeneration of the epidermis.[9] The most typical changes of the infection are evident in the nuclei of epidermal keratinocytes, where there is peripheral margination of chromatin attached to the nuclear membrane. As a consequence, the keratinocytes show ringed nuclei with a homogeneous ground-glass appearance combined with ballooning of the nucleus. The earliest noteworthy abnormality in the cytoplasm of these keratinocytes is the presence of vacuolization.[9]

The histopathologic differential diagnosis of HSV and VZV infections may be established immunohistochemically. Immunoperoxidase stains specific for HSV-1 (Fig. 1), HSV-2 (Fig. 2), and VZV (Fig. 3) are available commercially.[10,11] In all cases, the cells exhibit both nuclear and cytoplasmic staining, although staining is more intense at the edges of the infected nuclei, confirming the histopathologic observation of cells with peripheral margination of chromatin. The intensity of staining varies between cells depending on the type of infection. In infections by HSV-1 and HSV-2, epidermal keratinocytes show the strongest staining and only occasionally staining of the superficial portion of the follicular infundibulum can be observed. In early infections because of VZV, however, the strongest staining is seen in the cells of the outer root sheath of the hair follicle and in sebocytes of the sebaceous gland.[12,13] IHC can also demonstrate VZV antigens in nerves and other dermal structures. Epidermal keratinocytes, on the contrary, tend to be negative for VZV, at least in the early stages of infection.[13] Based on such immunohistochemical studies and the demonstration of viral antigens in various cutaneous structures, it has been possible to establish the sequence of herpes zoster lesion reactivation.[9] A histopathologic finding of folliculosebaceous involvement is characteristic of incipient erythematous lesions, whereas epidermal involvement and the formation of vesicles will be found in fully developed lesions.[9,13]

Clinicians and pathologists may also wish to use laboratory tests to establish definitive diagnosis in the case of atypical cutaneous manifestations of these viruses. For example, IHC was able to identify HSV infection in 5 bedridden geriatric patients (type 1 in 3 and type 2 in 2) with genital ulcers when histology was suggestive of HSV infection in only 2 of the 5 patients.[14,15] Another study showed that the sensitivity and specificity of IHC were comparable with in situ hybridization (ISH) in diagnosing HSV infections.[16] Similarly, IHC has also been shown to have higher specificity and sensitivity than standard microscopic assessments in diagnosing VZV infection through detection of VZV ORF63 encoded protein (IE63) and VZV late protein gE on both smears and formalin-fixed paraffin-embedded skin sections.[10] This can be of special significance in allowing early diagnosis of VZV infection in immunocompromised patients and thus early treatment. VZV infections may also

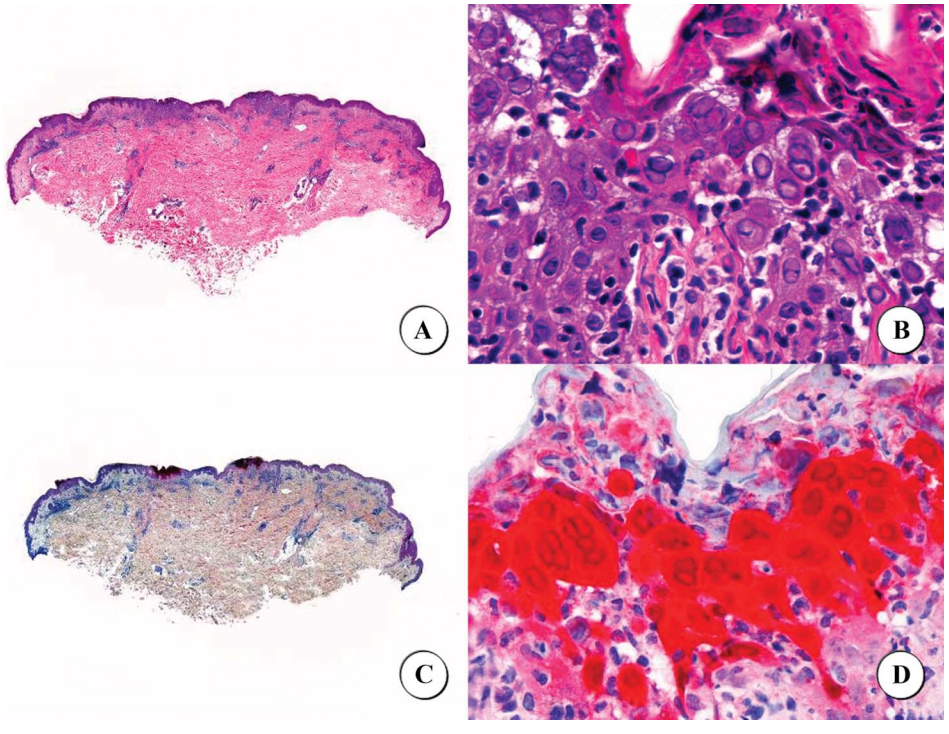

FIGURE 1. Histopathologic and immunohistochemical findings in a cutaneous infection by HSV-1 in the upper lip. A, Scanning power showing 2 foci of epidermal involvement and inflammatory infiltrate in the superficial dermis. B, Higher magnification demonstrated the characteristic cytopathic effect of herpesvirus infections in epidermal keratinocytes. C, The same case immunohistochemically studied for HSV-1 antibody. D, Positivity in the nuclei and cytoplasm of many epidermal keratinocytes. (A and B, hematoxylin–eosin stain; C and D, IHC for HSV-1. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

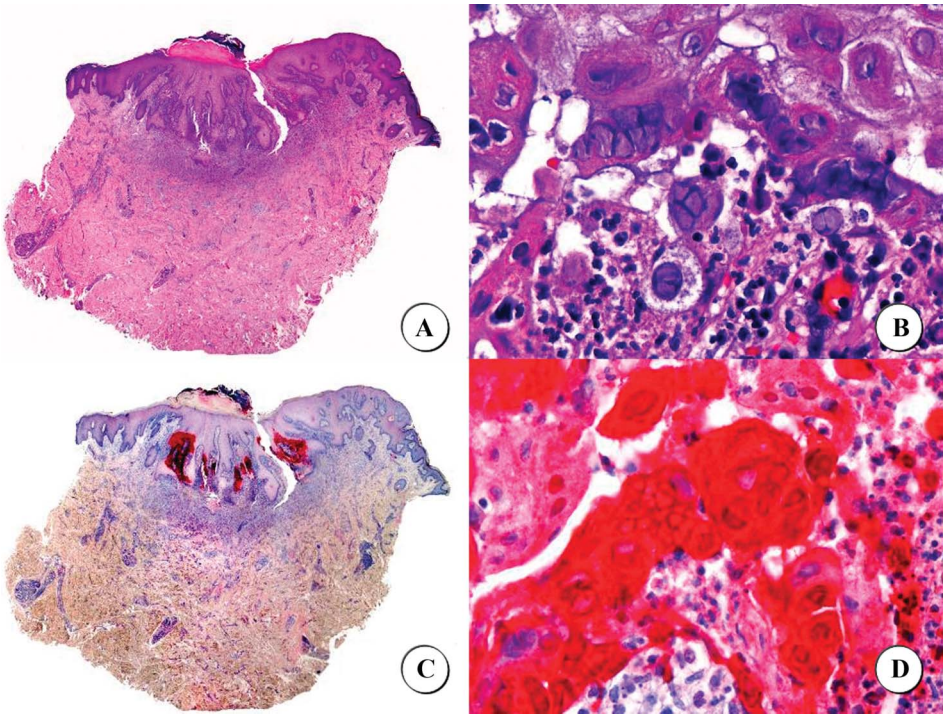

FIGURE 2. Histopathologic and immunohistochemical findings in a persistent cutaneous infection by HSV-2 in genital skin of a patient with AIDS. A, Scanning power showing irregular epidermal hyperplasia. B, Higher magnification demonstrated the characteristic cytopathic effect of herpesvirus infections in epidermal keratinocytes. C, The same case immunohistochemically studied for HSV-2 antibody. D, Positivity in the nuclei and cytoplasm of many epidermal keratinocytes. (A and B, hematoxylin–eosin stain; C and D, IHC for HSV-2. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

be differentiated with certainty from HSV infections using monoclonal antibodies against the VZV envelope glycoprotein gp1.[17]

Epstein–Barr Virus

EBV, or HHV-4, is a DNA γ-herpes virus. The DNA core is surrounded by an icosahedral nucleocapsid of 162 capsomers and the envelope. The EBV genome encodes for nearly 100 viral proteins. This genome exists in 2 forms: circular (episomal) and lineal. The episomal form is the one adopted in latency periods, whereas the lineal form is the one evidenced during the lytic phase in which EBV replicates.

EBV causes infectious mononucleosis, hairy leukoplakia in patients with AIDS, Lipschütz ulcer (ulcus vulvae acutum),

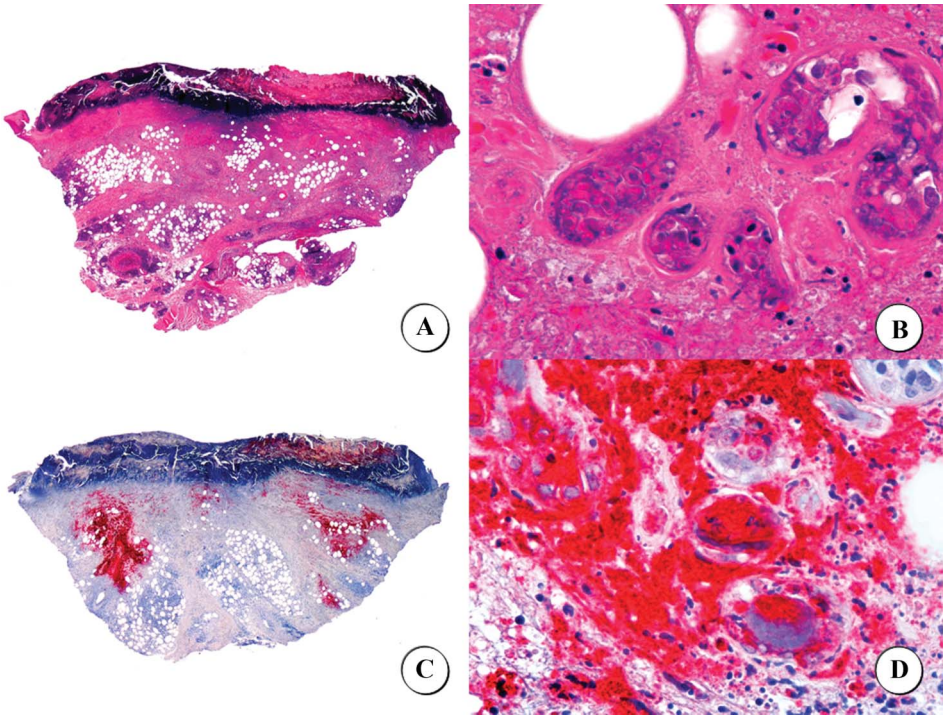

FIGURE 3. Figure 1. Histopathologic and immunohistochemical findings in a necrotic herpes zoster infection involving the lower back in a patient with AIDS. A, Scanning power showing epidermal necrosis and inflammatory infiltrate involving the upper and lower dermis. B, Higher magnification demonstrated necrotic eccrine coils. C, The same case immunohistochemically studied for VZV antibody showing several foci of positivity in the dermis. D, Positivity in the nuclei and cytoplasm of many necrotic keratinocytes of the eccrine coils. (A and B, hematoxylin–eosin stain; C and D, IHC for VZV. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

a variety of benign and malignant lymphoproliferative processes, and nasopharyngeal lymphoepithelial carcinoma.[9,17,18] In immunosuppressed patients, mainly HIV-positive and transplanted ones, an association between myoid tumors and EBV has been well established. The spectrum of EBV-associated myoid tumors includes leiomyoma, smooth muscle tumor of uncertain malignant potential, leiomyosarcoma, and myopericytoma. In general, EBV-related myoid tumors show some clinical peculiarities, such as being multifocal and appearing in uncommon locations.[19–28] EBV-associated leiomyosarcoma shows a more indolent biological behavior than classical leiomyosarcomas.[24]

In dermatology, it is also common to see a maculopapular rash in patients with infectious mononucleosis who have been treated with ampicillin, because of the presence of immunoglobulin (Ig) G or IgM antibodies against penicillin, but not IgE antibodies, as is the case in the hypersensitivity reaction to penicillin. As mentioned above, histopathologic findings in these rashes are nonspecific.

EBV displays a very high seroprevalence and is characterized by its latent persistence in memory B lymphocytes. The mere detection of EBV DNA therefore lacks diagnostic relevance. Switching from latent to replicative infection has only exceptionally been reported in B lymphocytes, and such a lytic phenomenon mainly occurs in the epithelia.

There are commercialized antibodies against latent membrane protein (LMP) 1 detected by conventional IHC and against Epstein–Barr nuclear antigen (EBNA) 2 detected by immunofluorescence, but these methods have relatively low detection sensitivity and accuracy. In contrast, identifying Epstein-Barr–encoded RNAs (EBERs) by ISH facilitates viral detection with high sensitivity and specificity and allows the visualization of viral RNA in the nucleus of tumor cells. At the present time, there are commercially available probes that can be used on paraffin-embedded tissue with a permanent chromogen. Therefore, ISH remains the gold standard for EBV detection in tissue samples[17] (Fig. 4). Western blot, flow cytometry, and enzyme-linked immunosorbent assay can potentially detect and measure selected viral proteins for which antibodies are available. However, the single most informative protein-based assay is ISH because it permits localization of protein in the context of histopathology, facilitating assessment of the medical significance of the infection.[29] Localization is achievable in paraffin-embedded sections for latent and lytic viral factors, including EBNA1, EBNA2, LMP1, LMP2, BHRF1, BZLF1, and BRLF1.[30] Interpretation of results, defining the spectrum of expressed genes and their localization to benign- or malignant-appearing cells, complements EBER ISH for diagnosis of EBV-related disease.[31] Immunostaining with EBV-LMP yields a cytoplasmic pattern.[12] This marker is useful in the diagnosis of posttransplant lymphoproliferative disorders, Hodgkin lymphoma, and other lymphomas. It is also positive in 25%–50% of nasopharyngeal carcinomas.[12] LMP1 immunostain (cytoplasmic and membranous localization) has been shown to be nearly as effective as EBER ISH in identifying EBV in lymph nodes of patients with infectious mononucleosis. Likewise, BZLF1 (characterizes lytic viral replication) immunostains (nuclear staining) have been shown to be useful in confirming diagnosis of oral hairy leukoplakia in tongue biopsies of AIDS patients.[32]

Technical problems can foil interpretation of immunohistochemical results. For example, EBNA1 is thought to be expressed in virtually all latently infected tumors; yet, EBNA1

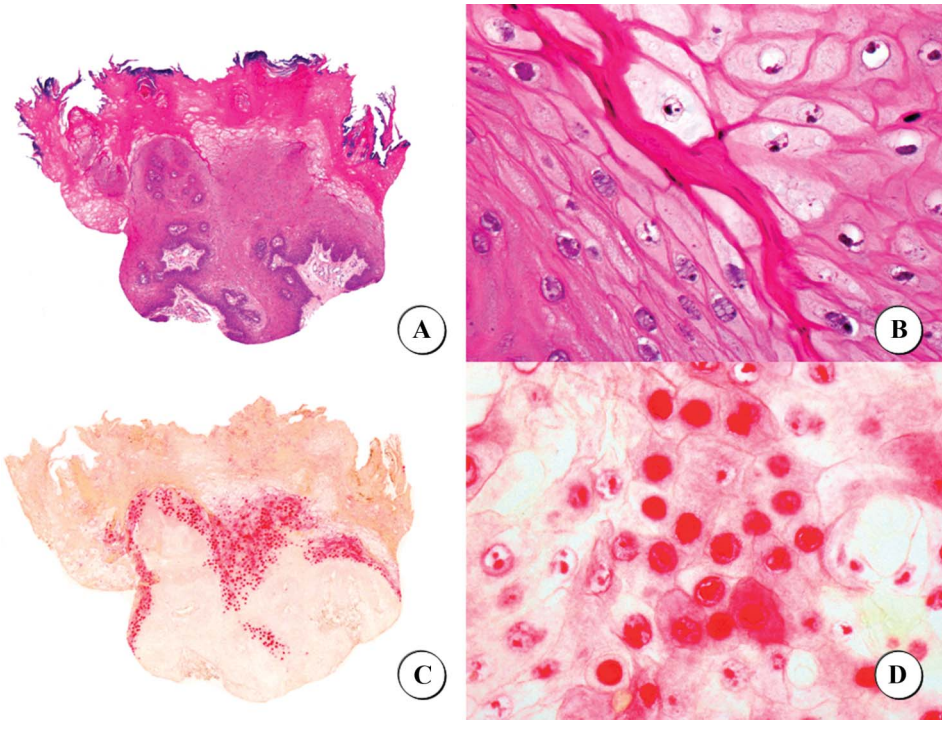

FIGURE 4. Histopathologic and ISH findings in a patient with hairy leukoplakia and AIDS. A, Irregular epithelial hyperplasia with ballooning of the keratinocytes of the upper layers. B, Higher magnification showing large pale-staining cells in the upper layers of the epithelium with irregular wrinkled nuclei. Note the presence of some intranuclear inclusion bodies. C, The same case studied by ISH for EBER. D, Many nuclei of the keratinocytes of the upper layers show EBER positivity. (A and B, hematoxylin–eosin stain; C and D, ISH for EBER. Original magnifications: A, ×10; B, ×400; C, ×10, D, ×400.)

IHC is not sensitive enough to reliably substitute for EBER ISH. Furthermore, the 2B4 clone of EBNA1 antibody cross-reacts with human MAGEA4, potentially causing false-positive interpretations.[33] Problems such as these emphasize the need to validate laboratory assays before they are used in clinical investigations.

Cytomegalovirus

CMV belongs to the subgroup of β-herpesviruses. Like other members of the family Herpesviridae, this virus produces primary infection, latent infection, and reinfection; however, its site of latency is not known. CMV infection is especially common in immune-compromised patients (such as HIV-infected patients), and the cutaneous manifestations can be quite variable, including ulcers, vesicles, papules, purpuric macules, verrucous lesions, prurigo nodularis–like lesions, and digital infarcts. In CMV infections, the characteristic observations are made in the endothelial cells of dermal blood vessels. The nuclei of these cells have large eosinophilic inclusions surrounded by a clear halo.[34] Less frequently, inclusions similar to those found in the nuclei of endothelial cells have also been described in the nuclei of fibroblasts and macrophages and also in the nuclei of the epithelial cells of eccrine ducts in CMV infections.[35]

CMV skin infection may be difficult to identify by clinical or histopathologic findings for various reasons. First, cutaneous CMV is rare, and the paucity of cases does not lend itself to microscopists having the opportunity to study a large number of cases. Second, the clinical manifestations of CMV infection vary, and there are no specific clinical findings that allow a clinician to suspect the right diagnosis. Third, the histopathologic findings of cutaneous CMV are typically present in scattered cells, and often, only a few cells have cytopathologic changes.[35,36] Therefore, immunohistochemical analysis may be needed to confirm the diagnosis in the setting of intense inflammation or in cases where only few cells are infected[35] (Fig. 5). IHC allows for rapid diagnosis; its sensitivity is higher than that of light microscopy and is comparable with that of culture and ISH.

Immunostaining with monoclonal anti-CMV antibody by immunoperoxidase methods gives a nuclear staining pattern in early-stage infections and a nuclear and cytoplasmic pattern in late-stage infections.[12] The anti-CMV antibody is known to bind with great avidity to CMV antigen synthesized in both early and late stages of infection.[37]

Human Herpesviruses 6 and 7

HHV-6 and HHV-7 are ubiquitous and have been associated with several cutaneous diseases, including roseola infantum, infectious mononucleosis, Rosai–Dorfman disease, pityriasis rosea, lichen planus, hypersensitivity reactions, graft-versus-host disease, and several other cutaneous manifestations, including various malignancies.[38] HHV-6 consists of 2 closely related yet distinct viruses, designated HHV-6 variant A and HHV-6 variant B. HHV-6 variant B is the etiological agent of the common childhood illness exanthema subitum, also known as roseola infantum or sixth disease of childhood. In adults, infection is seen primarily in immunocompromised individuals. In immunocompetent patients, the

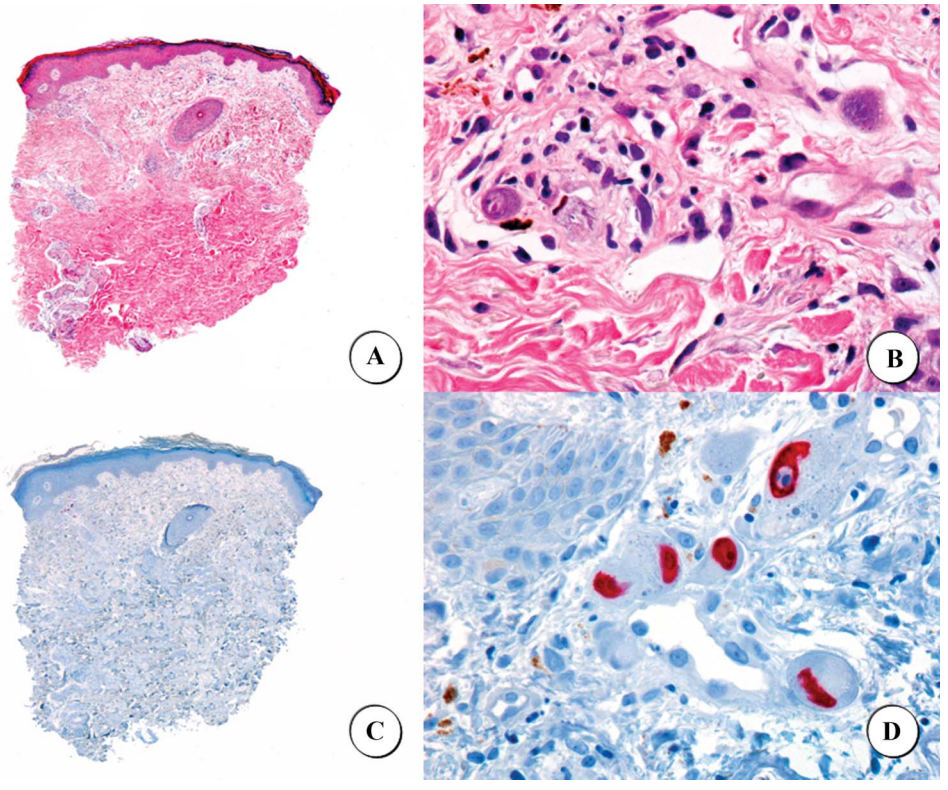

FIGURE 5. Histopathologic and immunohistochemical findings in the border of a perianal ulcer in a HIV-infected patient. A, Scanning power showing discrete perivascular infiltrates in superficial dermis and around eccrine units. B, Higher magnification demonstrated that the nuclei of some endothelial cells and perivascular cells contained large eosinophilic inclusions surrounded by a clear halo. C, The same case immunohistochemically studied for CMV. D, Immunoexpression for CMV in the cells containing eosinophilic inclusions in their nuclei. (A and B, hematoxylin–eosin stain; C and D, IHC for CMV. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

infection is usually diagnosed on the basis of its clinical features. However, the diagnosis in immunocompromised patients may need additional laboratory methods, including viral culture, serologic testing, polymerase chain reaction (PCR) assay, and tissue biopsy.[38] The histopathologic findings of HHV-6 and HHV-7 cutaneous infections are nonspecific. Epidermal spongiosis, some degree of vacuolar degeneration of the basal layer of the epidermis, and a superficial perivascular lymphocytic infiltrate may be observed.[9]

Immunohistochemical detection of HHV-6 and HHV-7 in skin biopsies is not commonly performed in routine clinical practice. However, several studies have reported positive results in the course of different clinical settings. Sumiyoshi et al[39] reported positive immunohistochemical staining with the HHV-6–specific monoclonal antibodies OHV-1 and OHV-2, using the avidin–biotin alkaline phosphatase method, in skin biopsy specimens from patients with primary HHV-6 infection and erythroderma. They found positive staining in the lymphocytes infected with HHV-6 in the skin.[39] Yadav et al[40] also reported the detection of HHV-6–associated antigens in formalin-fixed and paraffin-embedded oral tissues from patients with lichen planus, leukoplakia, and squamous cell carcinoma. They used the mouse (6A5G3) monoclonal antibody to the HHV-6 gp116/64/54 KDa component, which is a late protein in the viral replicative cycle. Furthermore, Broccolo et al[41] reported the immunohistochemical detection of the HHV-7 pp85 and the HHV-6 p41 antigens in skin biopsy specimens of patients with pityriasis rosea (17% and 67% of patients analyzed, respectively), suggesting that they might play an etiological role in this disease. HHV-7 and HHV-6 antigen-positive cells were located mainly in the perifollicular and papillary dermis, with the exception of 1 patient in whom HHV-7 pp85 was also found in the epidermal cells.

Human Herpesvirus 8

HHV-8 has been shown to be involved in the development of all the epidemiologic variants of KS. This involvement was demonstrated in 1994 when unique DNA sequences were isolated from biopsies of KS.[42] The virus, initially called KS-associated herpesvirus, was subsequently renamed HHV-8. HHV-8 infects the endothelial-derived spindle cells of KS and $CD19^+$ B cells. This latter event may be etiologically significant in the causation of some cases of multicentric Castleman disease and primary effusion lymphoma.[43] It has also been found in lymphomas and in other lymphoproliferative disorders with heterogeneous presentations.[44] Its presence in a number of skin cancers and in lesions of pemphigus vulgaris and pemphigus foliaceus has not been satisfactorily explained, although tropism for lesional skin has been postulated.[45,46]

HHV-8 can be detected by PCR in paraffin-embedded tissue, although the viral load seems to be low.[47,48] It is much easier to perform IHC using the commercially available monoclonal antibody against HHV-8 that is directed against the C-terminus of the latent nuclear antigen-1 (LNA-1) encoded by the ORF-73 gene.[49] The presence of HHV-8 in the nuclei of proliferating cells in KS lesions may be demonstrated in all its epidemiologic variants and from the earliest phases of the process. It is largely confined to the spindle cells in the nodular phase (Fig. 6), but it is found in the endothelial cells of the slit-like vessels in the early patch and plaque stages.

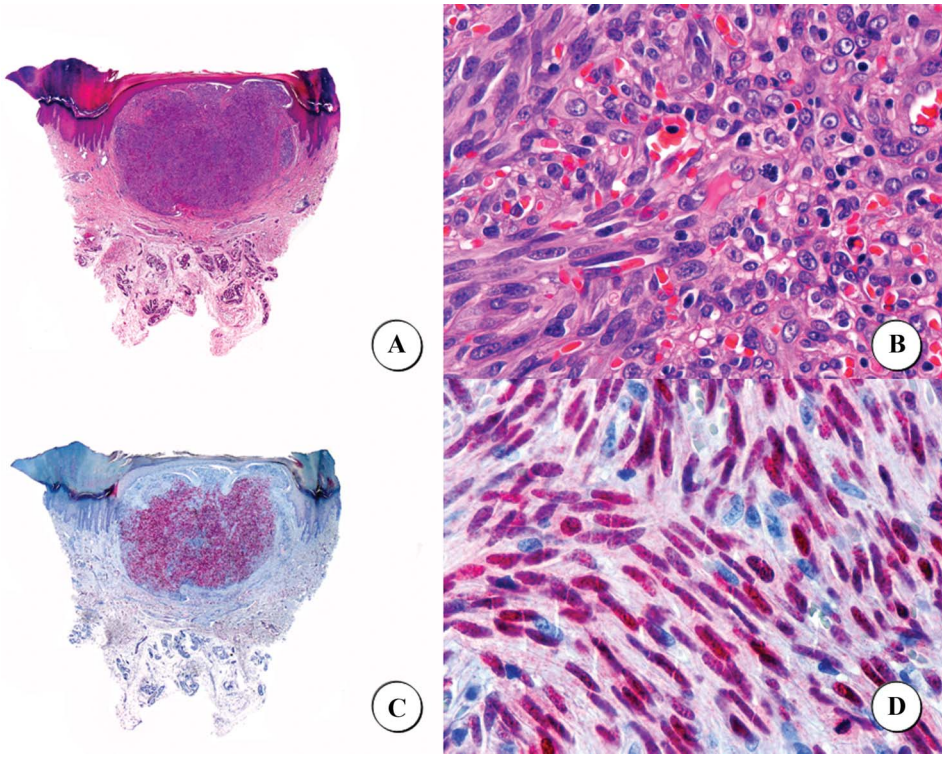

FIGURE 6. Histopathologic and immunohistochemical findings in a nodular lesion of an elderly male with classic KS. A, Scanning power showing a well-circumscribed dermal nodule. B, Higher magnification demonstrated short fascicles of spindled or rounded cells (depending on the cut plane) and small spaces filled by red cells. C, The same case immunohistochemically studied for HHV-8. D, Immunoexpression for HHV-8 in most of the nuclei of spindled cells. (A and B, hematoxylin–eosin stain; C and D, IHC for HHV-8. Original magnifications: A, ×10; B, ×400; C, ×10; D ×400.)

HHV-8 has both a lytic and latent phase of replication. During the latent phase, viral DNA copies are maintained as episomes attached to the host chromosome with the help of LNA-1.[50] This explains why LNA-1 immunoreactivity in KS cells most often appears as stippled nuclear staining. Most cells in KS lesions and HHV-8–infected cell cultures are latently infected. In only a small percentage (<5%) of infected cells is lytic HHV-8 replication seen.[51] This is clearly helpful for diagnostic purposes because most KS lesional cells will accordingly be LNA-1 immunoreactive.

Given the strong association between HHV-8 infection and KS, positive immunoreactivity for LNA-1 has proved to be the most useful diagnostic marker to help differentiate KS from its mimickers, including angiosarcoma, kaposiform hemangioendothelioma, spindle cell hemangioma, and benign lymphangioendothelioma, among others.[52,53] However, a minority of conventional angiosarcomas (29%) were found to be HHV-8 positive,[54] whereas no HHV-8 expression could be observed in radiation-related angiosarcomas.[55] LNA-1 IHC is favored over PCR (for HHV-8) for the evaluation of problematic vascular proliferations in patients infected with HIV. LNA-1 is a highly sensitive and specific immunohistochemical marker for KS in both the general (HIV negative) population and HIV-positive patients.[56] HHV-8 immunoreactivity seems to be unaffected by HIV status, patient age, gender, tumor recurrence, or the site of the KS lesion.[49]

Immunostaining KS with LNA-1 for diagnostic purposes is especially useful for the following: (1) Early subtle patch-stage lesions in which the histopathologic findings are not immediately obvious and may be difficult to diagnose on tissue biopsy. In these cases, inflammatory processes are the main differential diagnosis to be considered, stasis dermatitis being the most important. Other histopathologic alterations, such as ulceration, lymphedema, and secondary infections, may obscure the histopathologic characteristic of KS, rendering viral detection crucial for the diagnosis. (2) KS lesions that mimic other conditions, like pyogenic granuloma–like and angiomatous variants of KS. These histopathologic variants of KS are characterized by the proliferation of well-formed capillaries organized in lobules and surrounded by epidermal collarets, with no solid aggregates of fusiform cells, and they mimic benign vascular proliferations with a lobular capillary hemangioma pattern.[50] (3) KS presenting in unusual locations. (4) Histopathologic differential diagnosis with other vascular proliferations mimicking KS, such as hobnail hemangioma, spindle cell hemangioma, benign lymphangioendothelioma, and kaposiform hemangioendothelioma. The presence of HHV-8, however, seems not to be fully restricted to KS, as HHV-8 has been detected (albeit rarely) in some angiosarcomas, hemangiomas, and dermatofibromas,[57] although the meaning of this finding remains uncertain. Finally, the immunohistochemical demonstration of HHV-8 DNA may be a useful adjunct in the diagnosis of KS by fine-needle aspiration.[58]

PAPILLOMAVIRUSES

Papillomaviruses are DNA viruses, which replicate in the nucleus. The only important virus in this group in dermatopathology is the human papillomavirus (HPV), which produces various types of warts on different areas of the skin.[59] Several phylogenetic groups may be distinguished among the more than 150 known HPV types. Group A HPV types, occurring on genitals and mucous membranes, and cutaneous group B beta HPV, also associated with epidermodysplasia verruciformis, are of greatest significance for dermatologists.

The diagnosis of HPV-associated benign and malignant epithelial lesions is predominantly based on clinical features and conventional histopathology. Two of the most specific histopathologic findings are the presence of koilocytosis, which is not invariable, especially in long-standing warts, and the so-called koilocytotic atypia, mostly seen at the granular layer, which consists of nuclear variation in size and staining pattern, with irregularity of nuclear membrane and binucleated or multinucleated cells.[60] With only few exceptions (HPV types 4 and 63 with filamentous inclusion bodies[61] and HPV type 60 with eosinophilic intracytoplasmic large granules[62]), histology does not grant exact specification of the HPV type involved.[9] Papillomavirus antigen can be detected by immunoperoxidase methods.[63] Immunohistochemical detection of the HPV L1 capsid antigen (Fig. 7) is indicative of replicative HPV infections, but this method does not facilitate identification of the virus type involved, either. HPV antigens are detected in 64% of acanthopapillomas with koilocytes, but not in any lesion without histologic evidence for koilocytes, thus indicating that IHC is only of limited value.[64]

The use of new techniques, such as DNA hybridization, has allowed the separation of more than 120 antigenically distinct strains of HPV.[65,66] Further genotypes have been identified, but not fully characterized. PCR is now used routinely for the typing of HPV.[67] In recent years, attempts have been made to relate specific antigenic strains of HPV to particular clinicopathologic groups of verrucae.[68,69]

In the field of cervical cancer, the identification of HPV-related precancer that is likely to progress to an invasive carcinoma is very important. Although cervical cancer screening relies on cervical cytology and high-risk HPV detection, the histologic diagnosis, and specifically lesion grade, is the main parameter that drives clinical management of screen-positive women. Morphologically diagnosed squamous intraepithelial lesions regress spontaneously in more than half of the cases, but identifying those likely to persist and progress is not currently possible based on morphology. It has been suggested that patterns of viral protein expression may be used to differentiate between self-limited productive viral infection and a true precancer.[70] Some studies have evaluated the immunohistochemical expression of HPV capsid proteins L1 and L2 in squamous intraepithelial lesions as a predictive tool for progression.[71] Lack of major capsid protein L1 expression has been suggested as a feature in progressive lesions, whereas expression of the minor capsid protein L2 has not been extensively evaluated.

Recently, Wititsuwannakul et al[72] have evaluated the sensitivity and specificity of a commercially available anti-HPV antibody (BPV-1/1H8 + CAMVIR, ab 2417, Abcam),

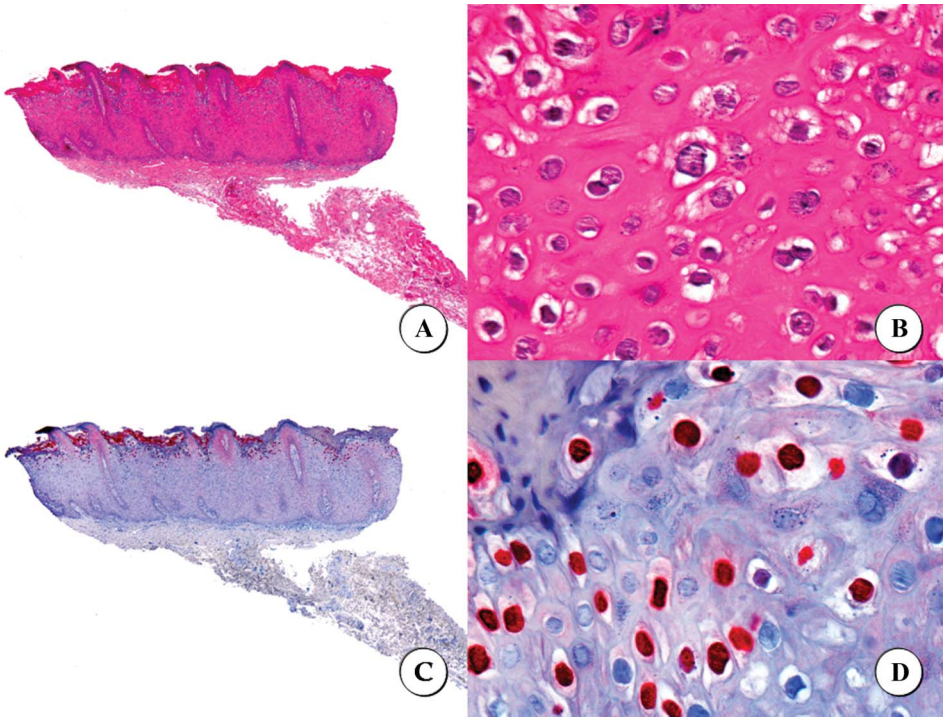

FIGURE 7. Histopathologic and immunohistochemical findings in a genital wart from an otherwise healthy young adult male. A, Scanning power showing a regular acanthosis of the epithelium. B, Higher magnification showed prominent koilocytosis in the upper layers of the epithelium. C, The same case immunohistochemically studied for HPV L1 capsid antigen. D, Immunoexpression for HPV L1 is seen in many nuclei of the keratinocytes of the upper layers. (A and B, hematoxylin–eosin stain; C and D, IHC for HPV L1. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

which detects HPV types 1, 6, 11, 16, 18, and 31 in formalin-fixed paraffin-embedded tissue, in 25 lesions (both HPV induced and non-HPV induced) mostly from the genital region, compared with ISH and hematoxylin and eosin staining. They found a sensitivity and specificity for this anti-HPV antibody of 90.9% and 85.7%, respectively, compared with ISH. IHC, like ISH, was generally positive in cases showing koilocytes/koilocytotic atypia (86%). However, IHC also detected productive infection with HPV in 23% (3 of 13) of cases without koilocytes/koilocytotic atypia. Positive immunostaining is mostly seen in corneocytes, the nuclei of the keratinocytes of the granular layer, and upper layers of the epidermis (Fig. 8). A

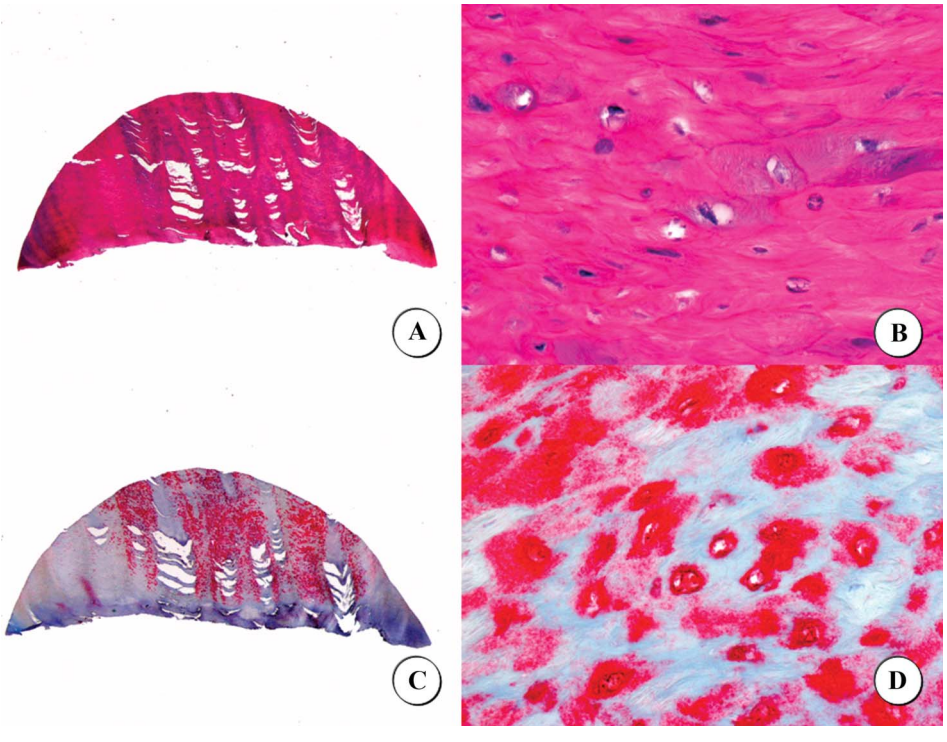

FIGURE 8. Histopathologic and immunohistochemical findings in a genital wart from a young adult woman with history of persistent condyloma acuminatum involving the vulva. A, Scanning power of a shave biopsy showed a thick compact orthokeratotic horny layer. B, Higher magnification showed some koilocytes in the upper layers of the horny layer. C, The same case immunohistochemically studied with CAMVIR antibody. D, Immunoexpression for HPV in many nuclei of the corneocytes of the upper layers with CAMVIR antibody. (A and B, hematoxylin–eosin stain; C and D, IHC for CAMVIR antibody. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

major disadvantage of IHC, compared with ISH and PCR, is its inability to type HPV. But IHC shows considerable advantages over ISH and PCR, such as the low cost, rapid turnaround time, and the ability to visualize productive HPV infection in tissue sections.[72] Because results of IHC for HPV with this antibody correlate with ISH, both tests are very helpful to confirm HPV infection (IHC) and subsequent HPV typing (ISH).

In conclusion, this review is focused on the use of IHC to identify viral pathogens that are relevant to dermatopathology and on the usefulness of these techniques in the diagnosis of cutaneous viral infections and other related diseases.

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CME EXAMINATION
JANUARY 2015

Please mark your answers on the ANSWER SHEET.

Upon completion of this learning activity, participants should be better able to use immunohistochemistry (IHC) to identify viral pathogens that are relevant to dermatopathology and apply these techniques in the diagnosis of cutaneous viral infections and other related diseases.

1. In cutaneous infections due to Herpes simplex virus, the strongest immunohistochemical staining is seen in the:

   a. Lymphocytes of the inflammatory infiltrate
   b. Apocrine glands epithelium
   c. Eccrine glands epithelium
   d. Outer root sheath of the hair follicle
   e. Epidermal keratinocytes

2. In cutaneous early infections due to Varicella-zoster virus, the strongest immunohistochemical staining is seen in the:

   a. Outer root sheath of the hair follicle
   b. Sebocytes of the sebaceous gland
   c. Epidermal keratinocytes
   d. Nerves of the dermis
   e. a and b

3. Epstein–Barr virus has been related to all the following diseases, except:

   a. Infectious mononucleosis
   b. Hairy leukoplakia in patients with AIDS
   c. Nasopharyngeal lymphoepithelial carcinoma
   d. Lymphoproliferative disorders
   e. Epidermodysplasia verruciformis

4. In Kaposi's sarcoma, with which of the following monoclonal antibodies will most lesional cells be immunoreactive?

   a. LNA-1
   b. VZV
   c. CM5E1
   d. Ab3
   e. LMP1

5. In which of the following viral-related skin diseases are the histopathological findings not specific?

   a. Trichodysplasia spinulosa
   b. Kaposi's sarcoma
   c. Exanthema subitum
   d. Orf
   e. Merkel cell carcinoma

6. Which of the following HPV-immunohistochemical staining patterns has been suggested as a feature indicating progression in the field of cervical cancer?

   a. Positive HPV-cytoplasmic immunoexpression
   b. Lack of expression of major capsid protein L1
   c. Lack of expression of major capsid protein L2
   d. Expression of the minor capsid protein L2
   e. Lack of expression of major and minor capsid proteins

ANSWER SHEET FOR THE AMERICAN JOURNAL OF DERMATOPATHOLOGY
CME PROGRAM EXAM
January 2015

Please answer the questions on page 12 by filling in the appropriate circles on the answer sheet below. Please mark the one best answer and fill in the circle until the letter is no longer visible. To process your exam, you must also provide the following information:

Name (please print): _____
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1. (A) (B) (C) (D) (E)
2. (A) (B) (C) (D) (E)
3. (A) (B) (C) (D) (E)
4. (A) (B) (C) (D) (E)
5. (A) (B) (C) (D) (E)
6. (A) (B) (C) (D) (E)

Your evaluation of this CME activity will help guide future planning. Please respond to the following questions below.

Please rate these activities (1 — minimally, 5 — completely)	1 2 3 4 5
These activities were effective in meeting the educational objectives	O O O O O
These activities were appropriately evidence-based	O O O O O
These activities were relevant to my practice	O O O O O

Please rate your ability to achieve the following objectives, both before and after this activity: 1 (minimally) to 5 (completely)

	Pre 1 2 3 4 5	Post 1 2 3 4 5
1. Use immunohistochemistry (IHC) to identify viral pathogens that are relevant to dermatopathology	O O O O O	O O O O O
2. Apply these techniques in the diagnosis of cutaneous viral infections and other related diseases	O O O O O	O O O O O

How many of your patients are likely to be impacted by what you learned from this activity?
O <20% O 20-40% O 40-60% O 60-80% O >80%

Do you expect that these activities will help you improve your skill or judgment within the next 6 months? (1 — definitely will not change, 5 — definitely will change)	1 2 3 4 5
	O O O O O

How will you apply what you learned from these activities (mark all that apply):

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In monitoring patients O	As a foundation to learn more O
In educating students and colleagues O	In educating patients and their caregivers O
As part of a quality or performance improvement project O	To confirm current practice O
For maintenance of board certification O	For maintenance of licensure O

How committed are you to applying these activities to your practice in the ways you indicated above? (1 — minimally, 5 — completely)	1 2 3 4 5
	O O O O O

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Immunohistochemistry in the Diagnosis of Cutaneous Viral Infections- Part II: Cutaneous Viral Infections by Parvoviruses, Poxviruses, Paramyxoviridae, Picornaviridae, Retroviruses and Filoviruses

Ana M. Molina-Ruiz, MD, Carlos Santonja, MD,† Arno Rütten, MD,‡ Lorenzo Cerroni, MD,§ Heinz Kutzner, MD,‡ and Luis Requena, MD**

Background: Cutaneous viral infections are increasing in recent years, particularly in immunocompromised patients.

Objective: Immunohistochemistry (IHC) provides a rapid and helpful tool that can be applied to confirm the diagnosis of specific viral infections that may be difficult to diagnose with certainty using routine microscopy alone.

Methods: Several immunostains that are useful in histopathology have been reviewed and tested in cutaneous samples of viral infections. Emphasis is placed on new stains and novel uses of existing stains.

Results: This article is an up-to-date overview of the potential uses of IHC in the histopathologic diagnosis of cutaneous viral infections by parvoviruses, polyomaviruses, poxviruses, paramyxoviridae, picornaviridae, retroviruses, and filoviruses.

Limitations: Specific monoclonal antibodies are commercially available only for some members of these virus families.

Conclusions: IHC may assist dermatopathologists to appropriately diagnose viral infections by parvoviruses, polyomaviruses, poxviruses, paramyxoviridae, picornaviridae, retroviruses, and filoviruses.

Key Words: immunohistochemistry, virus, skin, infection, parvoviruses, polyomaviruses, poxviruses, paramyxoviridae, picornaviridae, retroviruses, filoviruses

(*Am J Dermatopathol* 2015;37:93–106)

From the *Associate Professor of Dermatology and Dermatologist (Dr Molina-Ruiz), Chairman and Professor of Dermatology (Dr Requena), Department of Dermatology; †Pathologist, Department of Pathology, Fundación Jiménez Díaz, Universidad Autónoma, Madrid, Spain; ‡Dermatopathologist, Dermatophatologisches Gemeinschaftslabor, Friedrichshafen, Germany; and §Director of Dermatopathology and Professor of Dermatology, Department of Dermatology, Medical University of Graz, Austria.

All authors and staff in a position to control the content of this CME activity and their spouses/life partners (if any) have disclosed that they have no financial relationships with, or financial interests in, any commercial organizations pertaining to this educational activity.

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LEARNING OBJECTIVES

Upon completion of this learning activity, participants should be better able to:

1. Use immunohistochemistry (IHC) to identify viral pathogens that are relevant to dermatopathology.
2. Apply these techniques in the diagnosis of cutaneous viral infections and other related diseases.

INTRODUCTION

In this part II of this review, we highlight the available main immunohistochemical techniques that have been used and continue to evolve in the diagnosis of mucocutaneous infections caused by parvoviruses, polyomaviruses, poxviruses, paramyxoviridae, picornaviridae, retroviruses, and filoviruses.

MATERIALS AND METHODS

The biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. For routine histology, 5-μm-thick sections were stained with hematoxylin and eosin. Immunohistochemical stains were performed both at the Pathology Department of Fundación Jiménez Díaz University Hospital in Madrid and the Dermatopathology Laboratory at Friedrichshafen, Germany. Tissue sections were processed on a BioTek Solutions TechMate 500 (Dako), where 1-hour incubation with a primary antibody was performed. The immunohistochemical study of the viral pathogens was performed using the commercially available antibodies. Several antibodies for specific viruses described in the literature were not commercially available at the time of elaboration of this manuscript; therefore, immunohistochemical staining for these pathogens could not be tested.

PARVOVIRUSES

The Parvoviruses are single-stranded DNA viruses and among the smallest known DNA-containing viruses to infect mammalian cells. Parvovirus B19 (PVB19), which belongs to the genus *Erythrovirus*, is the only known human pathogen in this family.[1] Primary infection with PVB19 can be either asymptomatic or bring about a number of clinical syndromes. These include 2 specific PVB19-related dermatologic diseases, erythema infectiosum (fifth disease), and purpuric "gloves-and-socks" syndrome (PPGSS). The most frequently

observed dermatological manifestation of PVB19 infection is erythema infectiosum, while PPGSS is rare, and less than 100 cases have been reported.[2] The histopathologic findings in infectious erythema and PPGSS are nonspecific, and usually consist of foci of epidermal spongiosis, with a superficial perivascular infiltrate mostly composed of lymphocytes and some extravasated red cells.[2]

Demonstration of viral DNA in serum and/or skin biopsy samples has been carried out in a handful of reports as a means of linking PPGSS to PVB19 infection.[3–5] However, it has become clear that PVB19 can persist for a long time in human tissues,[6] and viral DNA has been found in normal skin with a frequency ranging from 22.5% to 76%.[7,8] This raises the issue whether PVB19 should be regarded as a true pathogen or as an innocent bystander. However, PVB19 can be detected immunohistochemically in the cytoplasm of endothelial cells of congested capillaries in the papillary dermis in skin lesions in PPGSS (Fig. 1) and infectious erythema using the anti-PVB19 monoclonal antibody directed against the viral protein VP2.[2,9,10] Therefore, immunohistochemical detection of PVB19 in the endothelial cells of biopsy specimens performed in the clinical setting of PPGSS and other related exanthemas could be pathogenetically significant and help in the understanding of these diseases.

PVB19 infects cells possessing the receptor for viral entry, the P blood group antigen globoside, with help from coreceptors alpha5beta1 integrin and Ku80.[11] The distribution of viral receptors in different tissues (erythroid progenitors, endothelium, megakaryocytes, fetal myocytes, liver, lung, kidney, synovium, and placental trophoblast) plays a significant role in the pathogenesis of the most frequent syndromes related to PVB19 infection, which include transient aplastic crises, anemia, fetal hydrops, fifth disease, PPGSS, and arthropathy. Santonja et al[2] demonstrated PVB19 endothelial positivity in several cases of PPGSS and proposed that the immunohistochemical detection of PVB19 in the endothelial cells of PPGSS suggests that PVB19 triggers an immunologic response responsible for the histopathologic findings of perivascular lymphocytic inflammation/lymphocytic vasculitis, edema, and red blood cell extravasation.[2] Moreover, involvement of the endothelium by PVB19 is in keeping with the expression of the viral receptor globoside by endothelial cells and suggests that the exanthema results from a cytotoxic T-cell reaction to virally infected cells. Other authors[9,10] have found PVB19 positive labeling not only of the endothelial cells of the dermal vessels but also of the epithelium of eccrine glands, ducts, and epidermal keratinocytes in biopsy specimens of PPGSS.

Endothelial cell involvement by the virus has also been investigated by PVB19 RNA expression in endothelium, by reverse transcriptase in situ polymerase chain reaction (PCR) and by immunohistochemistry (IHC) in the context of vasculitis and autoimmune diseases.[12] The subsequent immunologic reaction has been linked not only with dermatologic conditions (including scleroderma, adult Schönlein purpura, and dermatomyositis)[13–15] but also with systemic diseases, such as systemic lupus erythematosus, myocarditis, and pulmonary fibrosis.[16–18]

POLYOMAVIRUSES

Polyomavirus (PyV) infections were accidentally discovered in the 1950s when characterizing a transmissible agent causing multiple tumors in rodents, hence providing the name (Greek poly-multiple; -oma, tumors).[19] Today the expanding family of human PyV (HPyV) consists of 10 members, but only 2 of them are important for dermatopathologists: the recently identified Merkel cell polyomavirus (MCPyV) and trichodysplasia spinulosa polyomavirus (TSPyV). Serological

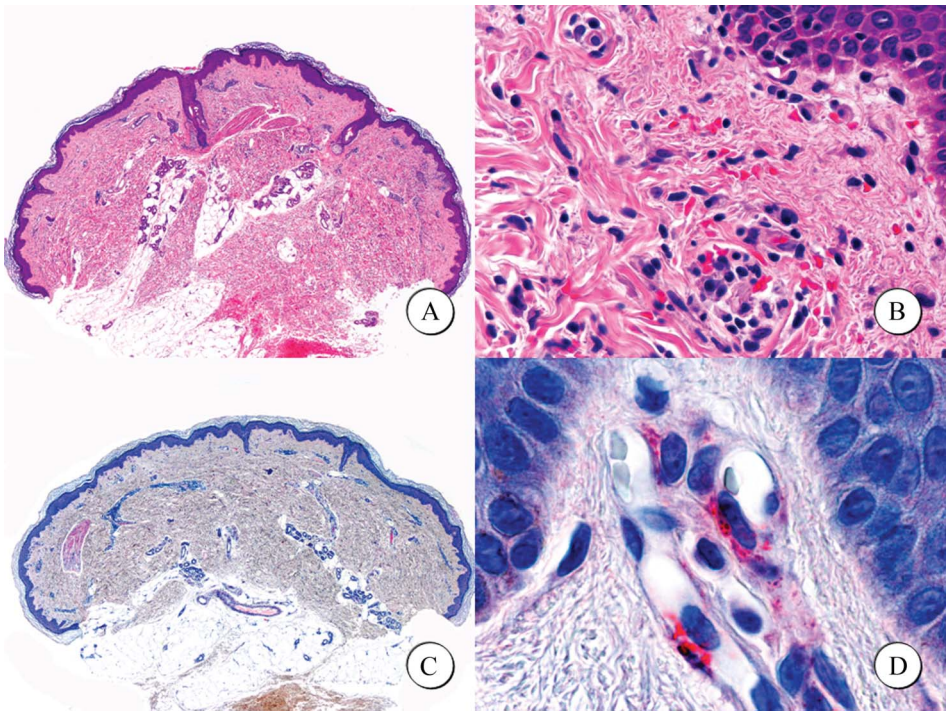

FIGURE 1. Histopathologic and immunohistochemical findings in the cutaneous lesions of a patient with PPGSS. A, Scanning power showing discrete perivascular infiltrates in the superficial dermis. B, Higher magnification demonstrated perivascular lymphocytes and extravasated red cells. C, The same case immunohistochemically studied with the anti-PVB19 monoclonal antibody directed against the viral protein VP2. D, Immunoexpression for PVB19 within the cytoplasm of endothelial cells (A and B, hematoxylin–eosin stain; C and D, IHC for PVB19 antibody; original magnifications: A ×10, B ×400, C ×10, and D ×400).

studies suggest that HPyVs subclinically infect the general population with rates ranging from 35% to 90%. However, significant disease is only observed in patients with impaired immune functions.[20]

Merkel Cell Polyomavirus

Merkel cell carcinoma (MCC) is a rare but aggressive human skin cancer that typically affects elderly and immunosuppressed individuals, a feature suggestive of an infectious origin. The search for an infectious agent yielded a major breakthrough in 2008; the discovery of the MCPyV by means of digital transcriptome subtraction.[21] Numerous studies rapidly validated the association of MCC with this virus that is integrated in the nuclei of MCC neoplastic cells.[22–24] Although the association between MCC and the MCPyV is now well established, it is also clear that this virus is not required for MCC as approximately 20% of these tumors contain no detectable MCPyV. Moreover, it is clear that MCPyV is not sufficient for developing MCC because it remains a rare cancer despite the fact that more than half of adults have antibodies to MCPyV and hence were exposed to the virus, typically in childhood.[24,25] Finally, MCPyV is not exclusive to MCC because it has also been observed rarely in certain squamous cell carcinomas in immunosuppressed patients.[26,27]

Since the original report, multiple studies have demonstrated the clonal integration of MCPyV in the nucleus of MCC cells in approximately 80% of the cases. A variety of methods have been used to detect the presence of the virus, including Southern blot analysis, PCR amplification of viral DNA, detection of integrated PyV sequences, in situ hybridization, DNA sequencing by hybrid capture, RT-PCR, and IHC with antibodies specific for MCPyV large T antigen and small T antigen.[24,28–30] Based on homology to other polyomaviruses, the MCPyV large and small T antigens are predicted to be oncogenic and contribute directly to the carcinogenesis of MCC.[30]

Shuda et al[29] developed a monoclonal antibody CM2B4 against a peptide fragment of the MCPyV T antigen exon 2 to detect MCPyV oncoprotein expression in the MCC tumor cells directly. The majority of the CM2B4-positive tumors showed strong and diffuse nuclear labeling in a large part of the tumor cell population (Fig. 2) and 77% of the PCR-positive tumors were immunoreactive for CM2B4. Later, these same authors[31] reported that the CM2B4 antibody failed to detect large T antigen in several cases of MCC but that many of these negative specimens stained positive with the monoclonal antibody CM5E1 specific for MCPyV small T antigen. The combined use of CM2B4 and CM5E1 led to detection of MCPyV T antigens in 47 (92%) of 51 cases of MCC, whereas CM2B4 alone detected large T antigen in 75% of cases.

Recently, Rodig et al[30] have performed IHC staining with a newly developed mouse monoclonal antibody, Ab3, that has markedly increased sensitivity in detecting MCPyV large T antigen (97%) in MCC compared with CM2B4 (80%). They propose that the presence of MCPyV in MCC is more common than previously reported and that improved detection methods may reveal that practically all MCC specimens contain viral DNA of MCPyV. Ab3 does not stain lymphocytes, even when used at higher concentrations or when staining tonsillar and reactive lymph node tissue specimens. The lack of nonspecific staining may be especially useful in clinical specimens because MCC tumors frequently contain infiltrating lymphocytes.[32] Furthermore, Ab3 did not stain tumor specimens from gastrointestinal neuroendocrine tumors or small cell lung cancer, supporting its usefulness as a clinical biomarker for MCC.

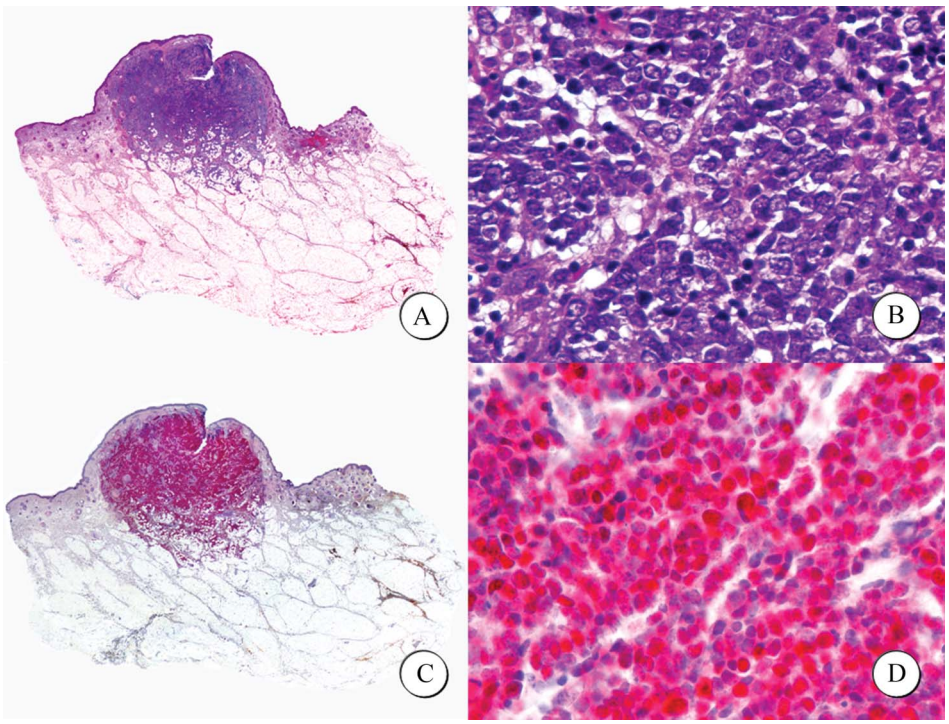

FIGURE 2. Histopathologic and immunohistochemical findings in a MCC. A, Scanning power showing a neoplasm that involved the full thickness of the dermis and extended to subcutaneous tissue. B, Higher magnification demonstrated that the tumor was composed of small round cells with vesicular nuclei and multiple small nucleoli. C, The same case immunohistochemically studied with the monoclonal antibody CM2B4 against a peptide fragment of the MCPyV. D, Immunoexpression for CM2B4 is seen in the nuclei of neoplastic cells (A and B, hematoxylin–eosin stain; C and D, IHC for CM2B4 antibody; original magnifications: A ×10, B ×400, C ×10, and D ×400).

Several studies[26,27] have shown that MCPyV DNA may be identified by PCR in up to 70% of nonmelanocytic cutaneous neoplasms in immunocompetent and immunosuppressed patients. Unlike MCC, these skin tumors exhibit no immunohistochemical evidence for intracellular viral antigens. Inflammatory cells may have transported viral DNA into peritumoral infiltrates as suggested by the fact that inflammatory $CD14^+$ $CD16^-$ monocytes constitute a reservoir for MCPyV.[33] When interpreting MCPyV detection, it is therefore essential to consider both molecular biology and immunohistochemical results.

Trichodysplasia Spinulosa Polyomavirus

Trichodysplasia spinulosa (TS) is a folliculocentric skin disorder that presents as keratotic spiny papules typically distributed over the ears and structures of the central face and less commonly involves the extremities, trunk, and scalp. Alopecia, usually most severely affecting the eyebrows and eyelashes, often accompanies the disorder. Haycox et al[34], in 1999, were the first authors using the term TS to describe friable follicular spinous lesions in a patient who received a combined renal/pancreas transplant. The authors showed, for the first time, the intracellular presence of virus particles with an appearance that was interpreted by them as consistent with viral particles of the Papovaviridae family. However, the original description of this disorder was published by Izakovic et al, in 1995, when they reported hair-like hyperkeratosis in patients with kidney transplants and considered the process as a new cyclosporine side-effect.[35] Since then, several cases have been reported in the literature[20,36–67] under different names, including viral-associated trichodysplasia,[39,43,49,52,60] trichodysplasia of immunosuppression,[45] virus-associated TS,[40] follicular dystrophy of immunosuppression,[37] cyclosporine-induced folliculodystrophy,[38] pilomatrix dysplasia,[36] and spiny hyperkeratosis (hair-like hyperkeratosis).[41] In 2010, van der Meijden et al[46] identified a new HPyV (TSPyV) in plucked facial spines of a heart transplant patient with TS, confirming the PyV etiology of this process.

The histopathologic findings are distinctive and consist of distorted and dilated anagen hair follicles filled by sheets of eosinophilic cells exhibiting the appearance of inner root sheath cells.[34,35] These cells, which contain abnormally large trichohyaline granules, abruptly cornify without the presence of a granular cell layer. The involved follicles, instead of the full keratinization toward a mature hair shaft, appear replete with sheets of nucleated cornifying cells that persist throughout most of the lower segment of the hair follicles. The observation was made that pathogenesis seemed to be related to immunosuppression, with the lesions resolving as immune function returned to normal.

Several tools, including scanning electron microscopy, PCR analysis of viral DNA, or immunohistochemical staining for the PyV middle T antigen, have been used to identify the pathogenic virus associated with TS. In the initial description of viral-associated TS, immunohistochemical stains demonstrated increased Ki-67 protein expression and negative staining for Papillomavirus.[34] At that time, the initial virus was assumed to be part of the Papovaviridae family, which has subsequently been split into the Papillomaviridae and Polyomaviridae families. Most studies in TS have identified TSPyV by electron microscopy,[39,40,42,43,49,54,55,57] PCR studies,[20,49,54,55,57,59,66] or immunofluorescence,[54] but very few attempts have been performed by IHC.[55,60] In the case described by Fischer et al,[55] immunohistochemical investigation for the HPyV middle T antigen with the SV40 antibody, which recognized the NH2 terminus within amino acids 83–128 of a large tumor antigen of SV-40 and JCV of PyVs, failed to detect the virus in lesional skin, renal allograft, and urine specimens.[55] However, Wanat et al[60] have recently described immunohistochemical positivity with SV-40 antibody for TSPyV in lesional skin of a patient with TS, demonstrating positive staining of the large eosinophilic cellular inclusions within keratinocytes composing the inner root sheath in all the follicles examined in the patient's samples, and we have corroborated these findings (Fig. 3). These positive cellular inclusions are visualized on both vertical and horizontal sections, and scanning electron microscopy confirmed that these inclusions contained small, icosahedral, regularly spaced, intracellular viral particles consistent with PyV.[60] These authors support that immunohistochemical staining for the middle T antigen or other viral proteins may be a useful tool for the identification of this disease process and a potentially more useful and practical way to establish the diagnosis compared with PCR assay or electron microscopy.[60] However, further cases are required to verify the utility of immunohistochemical staining because a negative result would not exclude the diagnosis.

Finally, routine testing with PCR assay, electron microscopy, or immunohistochemical stains is not necessary in all cases because routine histologic staining can be sufficient to make the diagnosis in cases of classic clinical presentations. Because the true incidence and clinical spectrum of this recently recognized disease is not known, these tools may be valuable in confirming the diagnosis in cases involving more subtle or atypical presentations. It is important that clinicians are aware of all the potential evaluations that can be performed and that they recognize the newly described HPyV as the infectious agent that is responsible for TS.

POXVIRUSES

The family Poxviridae is divided into many genera, of which the genus *Orthopoxvirus* includes vaccinia virus, variola virus, cowpox virus, and at least 6 other species, including monkeypox virus, camelpox virus, and raccoonpox virus.[68,69] Only 3 other genera cause human disease: the genus *Parapoxvirus* causing milker's nodule, orf, and sealpox[69–71]; the genus/subgenus *Molluscipoxvirus* resulting in molluscum contagiosum[72]; and the genus *Yatapoxvirus* resulting in tanapox. Skin infections caused by parapoxvirus have characteristic histopathology.

Orf (Ecthyma Contagiosum)

Orf (ecthyma contagiosum) is primarily a disease of young sheep and goats, involving the lips and perioral area.[1] It is caused by a poxvirus of the paravaccinia subgroup. Orf can be transmitted to humans by contact with infected animals. Lesions, which measure approximately 1–3 cm or more in diameter, develop most commonly on the hands and forearms. Spontaneous regression is usual approximately in 7–8 weeks.[1]

The diagnosis is based on case history (contact with infected animals) and clinical features.[73] Viral cultures,

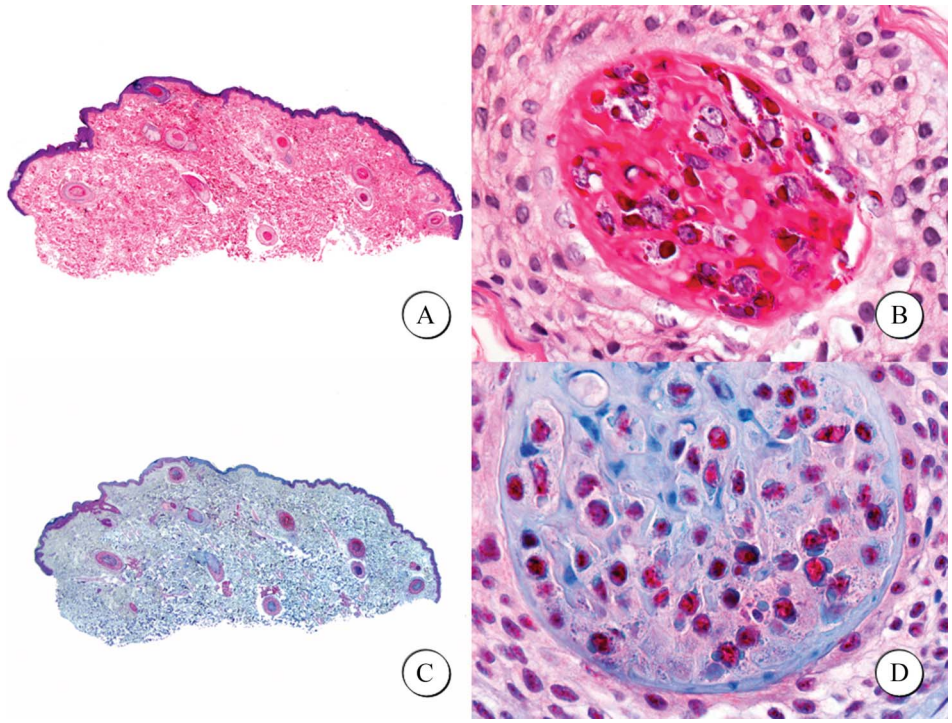

FIGURE 3. Histopathologic and immunohistochemical findings in cutaneous lesions of a patient with TS. A, Scanning power showing involvement of all hair follicles present in the biopsy. B, Higher magnification demonstrated that the inner root sheath cells contained abnormally large trichohyaline granules. C, The same case immunohistochemically studied with the monoclonal antibody SV-40 for TSPyV. D, Immunoexpression for SV-40 is seen in the large trichohyaline granules of the inner root sheath cells (A and B, hematoxylin–eosin stain; C and D, IHC for SV-40 antibody; original magnifications: A ×10, B ×400, C ×10, and D ×400).

histopathologic examination of biopsy specimens, electron microscopy, fluorescent antibody tests, and identification of specific viral nucleic acid have all contributed to establish the diagnosis.[73–76] Orf has a distinctive histological appearance of hyperkeratosis and cell ballooning in the cells of the upper layers of the epidermis. Eosinophilic intracytoplasmic inclusions are seen within these ballooned cells. The lesions of orf are generally regarded as histopathologically indistinguishable from milker's nodules,[77] although full-thickness epidermal necrosis seems to be more common in orf. Recently, a monoclonal antibody against the ORFV059 protein encoded by orf virus have been synthesized, which recognized the ORFV-Jilin ORFV059 protein in a variety of immunological assays, but to our knowledge, this antibody is not yet commercially available.[78]

PARAMYXOVIRIDAE

Measles

Measles is a highly contagious viral disease caused by an RNA virus of the Paramyxoviridae family, and human beings are the natural host and reservoir of infection. It spreads through the respiratory route and the virus tends to involve the lymph nodes, followed by viremia and infection of any organ, including the skin.[79] The disease is characterized by fever, malaise, cough, nasal congestion, conjunctivitis, and an erythematous maculopapular skin rash that appears 3 or 4 days after the onset of fever and spreads in a craniocaudal direction becoming confluent as the disease progresses. A pathognomonic enantem, named Koplik spots, appears during prodrome and consists of white papules on the oral mucosa. The disease is largely self-limiting in immunocompetent and immunized individuals but may be severe and even deadly in immunocompromised patients. All countries in the World Health Organization have renewed their commitment to eliminate measles transmission by 2015. However, measles has re-emerged in Europe recently because of suboptimal immunization levels that led to accumulation of susceptible populations over the last years.[80]

From an histopathologic point of view, the findings in skin rash of measles are usually nonspecific. Examination of the biopsy specimen usually shows basal vacuolar degeneration and mild dermal lymphocytic infiltrate, which usually is predominantly perifollicular. In some cases, multiple necrotic keratinocytes in the superficial epidermis, multinucleated intraepidermal cells, and pyknotic keratinocytes in the hair follicle epithelium have been described.[81,82] In the skin and oral mucous lesions (Koplik's spots), multinucleated keratinocytes with pyknotic nuclei have reported in the upper layers of the epithelium,[82,83] in the hair follicles,[84] and in the acrosyringium,[85] and they have been proposed as a cytopathologic clue for specific diagnosis. Intranuclear and intracytoplasmic inclusion bodies and abundant eosinophils in the infiltrate have been also described.[86]

Electron microscopic, immunofluorescence, and immunohistochemical studies have detected the measles virus in lesional skin within endothelial cells of dermal capillaries,[87] epidermal multinucleate cells,[83] dermal fibroblasts and macrophages,[87] lymphocytes,[84] keratinocytes of the upper layers of the epidermis,[84,88,89] hair follicles,[84,88,90] and sebaceous glands.[88] IHC detects the measles virus–specific antigens with a commercially available monoclonal antibody directed against the viral nucleoprotein[90] (Fig. 4). It has also been demonstrated that wild-type strains of measles virus use the signaling lymphocyte activation molecule (SLAM, also named CDw150) as a cellular receptor. In contrast, laboratory strains of measles virus and its derivate vaccine strains use both SLAM and the complement regulatory protein CD46 as cellular receptors.[91] Immunohistochemical studies of the skin of measles patients using monoclonal

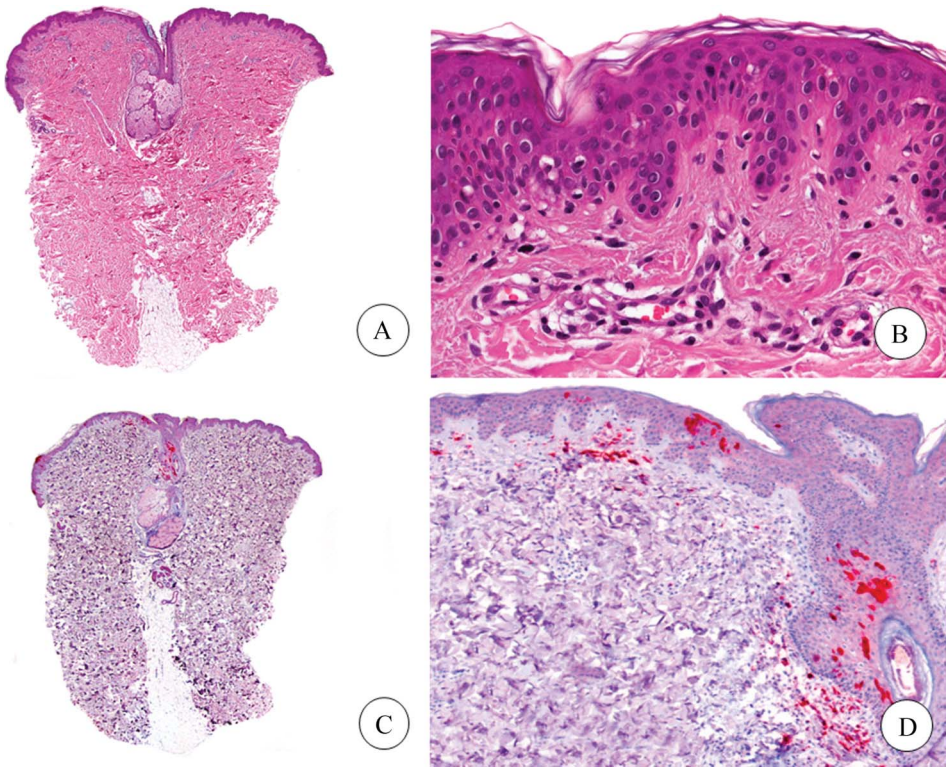

FIGURE 4. Histopathologic and immunohistochemical findings in the exanthema of patient with measles. A, Scanning power showing apparently normal skin. B, Higher magnification demonstrated isolated necrotic keratinocytes in the epidermis and mild superficial perivascular lymphocytic infiltrate. C, The same case immunohistochemically studied with the antibody for measles virus. D, Immunoexpression is seen in the nuclei of epidermal keratinocytes, keratinocytes of the hair follicle, and lymphocytes of the dermal infiltrate (A and B, hematoxylin–eosin stain; C and D, IHC for measles antibody; original magnifications: A ×10, B ×400, C ×10, and D ×200).

antibodies anti-SLAM and anti-CD46 have shown that hair follicles, epidermis, capillary blood vessels, fibroblasts, mononuclear cells, and acrosyringium were CD46 positive and SLAM negative.[90,92] These results suggest that CD46 may be a receptor of the wild measles virus in the skin.[90,93]

PICORNAVIRIADE

Enteroviruses

Enteroviruses comprise a subgroup of *Picornaviridae* family that cause a wide spectrum of disorders associated with exanthemas. The non-polio enteroviruses include echoviruses and coxsackie types A and B. These viruses have a single-stranded RNA genome and an unenveloped capsid.

Enteroviral infections are worldwide infections transmitted by fecal–oral or respiratory route. Enteroviruses first infect epithelial cells of the upper airway or lower alimentary tract, replicate in lymph nodes, and disseminate by an initial viremia. Subsequently, replication occurs in many organs and during this replication period is when clinical manifestations appear. A second and major viremia occurs during this period or viral replication occurs in the secondary infection sites.[94]

Hand-foot-and-mouth disease (HFMD) is the most characteristic exanthematous disease caused by enteroviruses. Numerous coxsackie serotypes have been implicated, coxsackie A16 being the most common etiologic factor. Prodromos consist of fever and malaise and few days later the exanthema appears. Dermatologic manifestations are characterized by the abrupt onset of elongated ovoid small vesicles on the palms and soles, in conjunction with erosive stomatitis. In rare cases, vesicles may also involve dorsum of the hands and feet and other nonacral areas of the skin. Most patients with HFMD have a benign and self-limited course, although in rare cases of outbreaks of severe enterovirus 71 infection, cardiopulmonary and neurologic complications and even death of involved patients have been described.[95]

Herpangina is a pediatric febrile disease cause by coxsackie virus groups A and B or echoviruses characterized by painful vesicles and erosions in buccal mucosa, soft palate, and tonsils.[96] Enteroviruses may also be the etiologic agent for the so-called eruptive pseudoangiomatosis, which consists of spontaneously regressing vascular papules composed of telangiectatic vessels, but without a proliferative component.[97,98]

Histopathologically, HFMD lesions show intraepidermal vesicles with prominent reticular degeneration and a few ballooned cells. No multinucleate cells or inclusion bodies have been described in lesions of HFMD. Usually, there is accompanying papillary dermal edema and a mild perivascular inflammatory infiltrate mostly composed of lymphocytes. Electron microscopic studies demonstrate viral particles.[99] From an immunohistochemical point of view, there is a commercially available antibody that reacts with an epitope on the VP1 peptide, which is highly conserved within the enterovirus group.[100] This antibody reacts with a molecule of 34–37 kDa molecular weight, and it was generated by using coxsackie B5 as immunogen. It reacts with most enteroviruses of echovirus, coxsackie and poliovirus group, but does not react with rotavirus, yellow fever virus, measles, rhinovirus A1, adenovirus 18, or hepatitis A virus.[101] In our experience, this generic anti-enterovirus antibody stains mostly keratinocytes of the acrosyringium in lesional skin (Fig. 5).

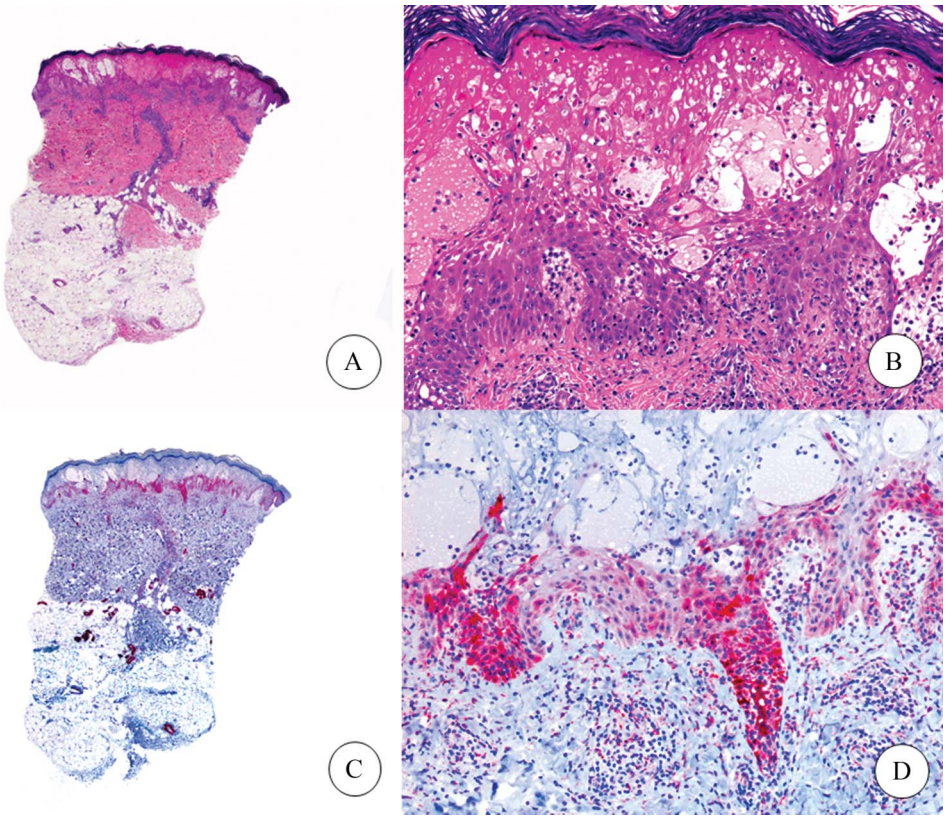

FIGURE 5. Histopathologic and immunohistochemical findings in cutaneous lesions of a patient with HFMD. A, Scanning power showing severe epidermal involvement. B, Higher magnification demonstrated intraepidermal vesicle with prominent reticular degeneration and a few ballooned cells. C, The same case immunohistochemically studied with the monoclonal antibody that reacts with the VP1 peptide of the enterovirus group. D, Immunoexpression for VP1 peptide of the enterovirus group is seen in epidermal keratinocytes and acrosyringeal keratinocytes (A and B, hematoxylin–eosin stain; C and D, IHC for enterovirus antibody; original magnifications: A ×10, B ×40, C ×10, and D ×200).

RETROVIRUSES

Human T-Cell Lymphotropic Virus Type 1

Human T-cell lymphotropic virus type 1 (HTLV-1), also named human T-cell leukemia virus type 1 and adult T-cell lymphoma virus type 1, is endemic in the south of Japan and in the Caribbean Islands and rare in other regions. Transmission of HTLV-1 occurs primarily by sexual intercourse, blood transfusions, needle sharing among intravenous drug abusers, and breastfeeding. HTLV-1 induces T-cell proliferation, followed by interleukin (IL)-2 receptor exposition, increased IL-2 secretion, and induction of interferon-γ, IL-5, and IL-10.

HTLV-1 is associated with infective dermatitis and adult T-cell leukemia/lymphoma (ATLL). Infective dermatitis is an exudative and eczematous dermatosis that involves mostly the scalp, axillae, groin, and small folds of the face.[102] In some cases, infective dermatitis may progress to more severe HTLV-1–associated disease, such as ATLL. Four variants of ATLL are recognized in the new World Health Organization classification of tumors of hematopoietic and lymphoid tissues[103]: acute and chronic leukemic, lymphomatous, and smoldering types. Although cutaneous manifestations are usually seen in the smoldering form of the disease, it has been suggested that patients with purely cutaneous lesions may have a better prognosis and should be classified separately from those with smoldering ATLL.[104] Cutaneous manifestations and histopathologic features of ATLL are identical to those of mycosis fungoides, so demonstration of retroviral infection is mandatory for diagnosis. Molecular analyses show a monoclonal rearrangement of the TCR gene and the presence of the integrated genome of HTLV-I.[105] In the early phase of the ATLL, neoplastic cell properties dependent on the HTLV-1 proviral DNA pX region p40Tax protein (Tax)[101–109] appear under the effects of external and internal mutagens[110–112] following the accumulation of mutations induced by repeated Tax expression[113] in the background of persistent proliferation of HTLV-1–infected T cells, which is probably induced by HTLV-1 basic leucine zipper (HBZ) messenger RNA.[114,115] Then, late-phase ATLL cells with neoplastic properties independent of Tax appear when mutation has progressed in the host cell DNA and HTLV-1 proviral DNA, including pX p40Tax DNA sequence.[110] Histopathological diagnosis of ATLL is expected based on IHC detecting of Tax, HTLV-1 proviral DNA pX p27Rex protein (Rex), or HBZ messenger RNA/protein in neoplastic cells[116] (Fig. 6).

FILOVIRUSES

The family Filoviridae is an unique negative-stranded RNA virus family that forms filamentous virions. Not much is known about filoviruses because their highly pathogenic nature makes them difficult to study. Two members of the family that are commonly known are Ebola virus and Marburg virus.[117] Both viruses, and some of their lesser known relatives, cause severe disease in humans and nonhuman primates in the form of viral hemorrhagic fevers.

Ebola Virus

Ebola virus causes a severe and often fatal hemorrhagic fever.[117] Ebola hemorrhagic fever (EHF) is a rare disease, and

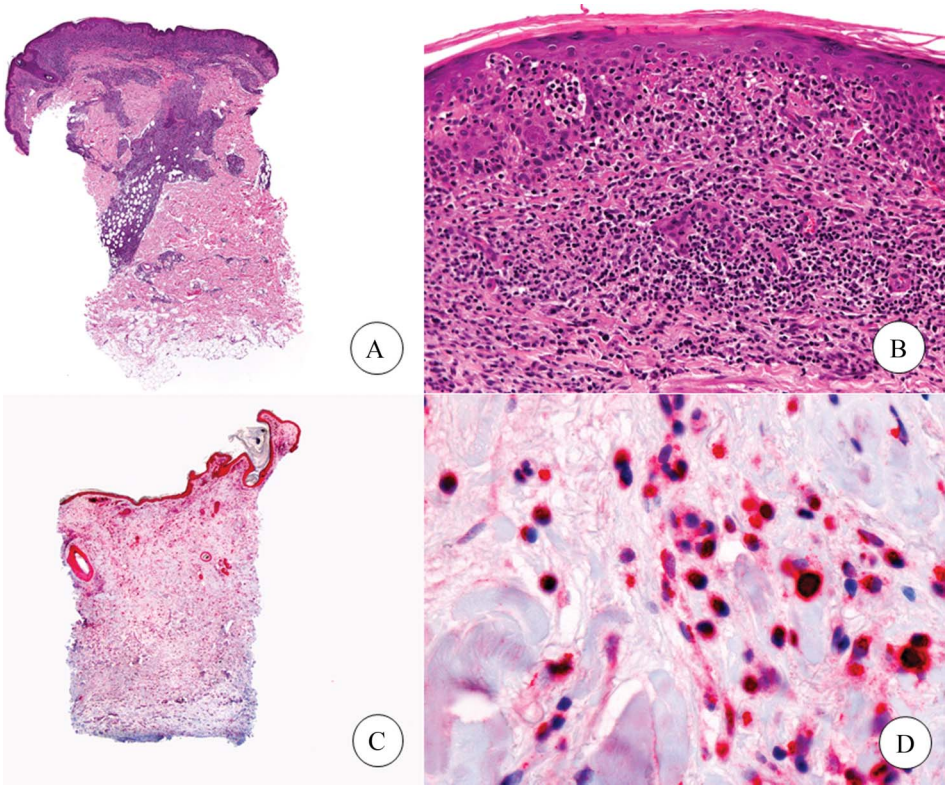

FIGURE 6. Histopathologic and immunohistochemical findings in cutaneous lesions of a patient with ATLL. A, Scanning power showing a dense band-like infiltrate in the superficial dermis and dense perivascular and perifollicular infiltrate. B, Higher magnification demonstrated the epidermotropism of the infiltrate in some areas. C, The same case immunohistochemically studied with the monoclonal antibody against HTLV-1. D, Immunoexpression for HTLV-1 is seen as a dot-like paranuclear positivity, probably located in Golgi apparatus of neoplastic lymphocytes. Large positive cells are mast cells expressing nonspecific immunostaining (A and B, hematoxylin–eosin stain; C and D, IHC for HTLV-1; original magnifications: A ×10, B ×40, C ×10, and D ×400).

the number of sporadic cases occurring in tropical Africa is unknown. The clinical diagnosis of EHF is often presumptive, and laboratory confirmation is essential. Traditionally, the laboratory diagnosis of EHF has been accomplished through virus isolation or serologic assays.[118,119] Because of the biosafety hazards associated with the handling and testing of EBO virus, these assays can be performed in only a few specialized laboratories worldwide, requiring transportation of the dangerous biological specimens from remote sites to these laboratories.

In 1999, Zaki et al[120] reported the development of a novel, safe, sensitive, and specific diagnostic immunohistochemical test for Ebola virus infections, which uses formalin-fixed postmortem skin specimens, and should be useful for EHF surveillance and prevention. This antibody is not commercially available. These authors evaluated skin biopsies of 14 patients during the EHF outbreak in the Democratic Republic of Congo in 1995. They found abundant viral antigens and particles within endothelial cells, mononuclear phagocytic cells, and fibroblasts in the skin of EHF patients using IHC, thus suggesting an epidemiologic role for contact transmission. Histopathologic changes in the skin tissue were not pathognomonic and consisted mainly of various degrees of endothelial cell swelling and necrosis.

CONCLUSIONS

IHC is an excellent diagnostic technique with the distinct advantage of being able to exactly locate a given protein within the tissue examined. The field is continuously expanding, with new applications steadily increasing. Ultimately, the possibilities of IHC in the field of cutaneous viral infections are immense, and the future is very promising.

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CME EXAMINATION
FEBRUARY 2015

Please mark your answers on the ANSWER SHEET.

Upon completion of this learning activity, participants should be better able to use immunohistochemistry (IHC) to identify viral pathogens that are relevant to dermatopathology and apply these techniques in the diagnosis of cutaneous viral infections and other related diseases.

1. Which of the following entities are PVB19-related dermatologic diseases?

   a. Erythema infectiosum (fifth disease)
   b. Roseola infantum
   c. Purpuric “gloves-and-socks” syndrome (PPGSS)
   d. b and c
   e. a and c

2. To which of the following diseases has Merkel cell polyomavirus been related?

   a. Squamous cell carcinoma
   b. Merkel cell carcinoma
   c. Melanoma
   d. a and b
   e. b and c

3. Immunohistochemical studies have detected the measles virus in lesional skin within endothelial cells of:

   a. Dermal capillaries
   b. Epidermal multinucleate cells and lymphocytes
   c. Keratinocytes of the upper layers of the epidermis
   d. Hair follicles and sebaceous glands
   e. All of the above

4. Hand-foot-and-mouth disease (HFMD) is an exanthematous disease caused by:

   a. Herpes viruses
   b. Papillomaviruses
   c. Poxviruses
   d. Enteroviruses
   e. Parvoviruses

5. Which of the following monoclonal antibodies has proved higher sensitivity in detecting MCPyV large T antigen when used in skin biopsy specimens?

   a. CM2B4
   b. LNA-1
   c. CM5E1
   d. Ab3
   e. LMP1

6. Trichodysplasia spinulosa has been recently associated with a new virus included in the family of:

   a. Herpesviridae
   b. Papillomaviridae
   c. Polyomaviridae
   d. Parvoviridae
   e. Poxviridae

ANSWER SHEET FOR THE AMERICAN JOURNAL OF DERMATOPATHOLOGY
CME PROGRAM EXAM
February 2015

Please answer the questions on page 104 by filling in the appropriate circles on the answer sheet below. Please mark the one best answer and fill in the circle until the letter is no longer visible. To process your exam, you must also provide the following information:

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1. (A) (B) (C) (D) (E)
2. (A) (B) (C) (D) (E)
3. (A) (B) (C) (D) (E)
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5. (A) (B) (C) (D) (E)
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Please rate your ability to achieve the following objectives, both before and after this activity: 1 (minimally) to 5 (completely)

	Pre 1 2 3 4 5	Post 1 2 3 4 5
1. Use immunohistochemistry (IHC) to identify viral pathogens that are relevant to dermatopathology	O O O O O	O O O O O
2. Apply these techniques in the diagnosis of cutaneous viral infections and other related diseases	O O O O O	O O O O O

How many of your patients are likely to be impacted by what you learned from this activity?
O <20% O 20-40% O 40-60% O 60-80% O >80%

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	O O O O O

How will you apply what you learned from these activities (mark all that apply):

In diagnosing patients O	In making treatment decisions O
In monitoring patients O	As a foundation to learn more O
In educating students and colleagues O	In educating patients and their caregivers O
As part of a quality or performance improvement project O	To confirm current practice O
For maintenance of board certification O	For maintenance of licensure O

How committed are you to applying these activities to your practice in the ways you indicated above? (1 — minimally, 5 — completely)	1 2 3 4 5
	O O O O O

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Carlos Santonja C, Ángel Santos-Briz, Gabrielle Palmedo, Heinz Kutzner, Luis Requena

Detection of human parvovirus B19 DNA in 22% of 1,815 cutaneous biopsies of a wide variety of dermatologic conditions suggests viral persistence after primary infection and casts doubts on its pathogenic significance

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Detection of human parvovirus B19 DNA in 22% of 1,815 cutaneous biopsies of a wide variety of dermatologic conditions suggests viral persistence after primary infection and casts doubts on its pathogenic significance

RUNNING HEAD: Parvovirus B19 in dermatologic conditions

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What's already known about this topic? Many dermatologic diseases have been linked to Parvovirus B19 infection due, among other evidence, to presence of viral Deoxyribonucleic acid (DNA) in tissue specimens.

What does this study add? Polymerase chain reaction (PCR) detection of viral DNA in 22% of 1,825 tested cases confirms data of previous shorter series and casts doubts on a pathogenic role for Parvovirus B19 in these conditions.

Summary

Background: Human parvovirus B19 (B19V) has been associated with a number of dermatologic and systemic conditions, including myocarditis and autoimmune syndromes.

Objectives: To determine the frequency of B19V deoxyribonucleic acid (DNA) detection in a large dermatopathology practice, and to characterize the histopathologic patterns involved.

Methods: We selected for polymerase chain reaction (PCR) detection of B19V a total of 1,815 skin biopsies pertaining to entities allegedly related to Parvovirus B19, as well as cases suspected clinically of representing paraviral exanthemas. Immunohistochemical detection of B19V viral protein 2 (VP2) was performed in 92 PCR-positive cases.

Results: B19V DNA was found by PCR in 402 out of 1,825 biopsy specimens (22%). VP2 protein was identified by immunohistochemistry only in three instances of papular purpuric "gloves and socks" syndrome.

Conclusions: Since the virus has the capacity to persist in different tissues (including the skin) for long periods, it could represent merely an innocent bystander, so no pathogenetic significance can be inferred from the PCR positivity for B19V in the vast majority of dermatologic conditions studied.

Keywords Parvovirus B19 Immunohistochemistry Dermatologic disorders Skin PCR

Introduction

Human parvovirus B19 (B19V), the best known parvovirus able to infect humans,[1,2] is responsible for a variety of diseases, including fifth disease (erythema infectiosum), cases of non-immune fetal hydrops, aplastic crises in patients with increased erythropoiesis, anemia in immunodeficient and immunocompromised patients, and acute or chronic arthropathy.[3] Three genotypes of B19V are recognized, differing in over 10% of their genome:[2] genotype 1 (by far worldwide predominant), genotype 2 (apparently restricted to subjects born before 1973), and genotype 3 (detected in West Africa and in only a few Western countries). The virus was first described in 1975 in the serum of nine healthy blood donors, a patient with acute hepatitis and another with a recent renal transplant, as false-positive results during routine screening for hepatitis B virus surface antigen.[4] In 1983 it was found out that B19V is the cause of erythema infectiosum.[5] In primary infection in adults, four clinical dermatologic patterns (exanthema, "gloves and socks", periflexural and palpable purpura) have been recently delineated;[6] these can rarely appear in combination.[7] On the other hand, a role for B19V has been proposed in about 40 dermatologic conditions. These range from uncommon entities with a strong etiological association to B19V, like papular-purpuric "gloves and socks" syndrome (PPGSS)[8] to classical dermatologic diseases with only an *a priori* remote relation with the virus. These include Henoch-Schönlein purpura, erythema nodosum, erythema multiforme, palmar desquamation/keratolysis exfoliativa, dermatomyositis-like rash, acute livedo reticularis, chronic urticaria, systemic sclerosis, chronic and acute pityriasis lichenoides, psoriasis, Behçet's disease, granuloma annulare, necrotizing vasculitis, Wells syndrome, Melkerson-Rosenthal syndrome, hydroa vacciniforme, acute exanthematous pustulosis, Sweet's syndrome and others.[9-38]This association has been mostly based on

serological proof of recent infection (i.e., IgM followed by IgG positive serology), or on the detection of viral DNA by PCR in serum or skin biopsies.[10,17,20,23,25,38,39] Apart from erythema infectiosum, viral protein or RNA in skin biopsies has only rarely been demonstrated by immunohistochemistry and/or reverse transcriptase-polymerase chain reaction (RT-PCR).[17,40,41]

The mere detection by PCR of viral DNA in a tissue sample has been challenged as proof of a definitive causal role for B19V, since PCR investigation has yielded positive results in normal skin, chronic urticaria, and a number of disparate dermatological conditions.[25,27,42-45] For this storage mechanism for the virus, the term *bioportfolio* has been proposed.[42] This suggests that B19V is in most settings but an innocent bystander, and questions its pathogenic role not only in dermatological diseases, but also in a number of medical conditions that along the years have been associated with it, like myocarditis.[46] We have sought to explore the frequency at which B19V DNA can be found by PCR in formalin-fixed, paraffin-embedded tissue of skin biopsies of a wide variety of cutaneous disorders in a large dermatopathology practice. With this aim, we undertook PCR study of B19V DNA in 1,815 routine and consultation dermatopathology cases, with selection criteria based on data from the published dermatologic literature on the subject. The gamut of clinically suspected dermatologic conditions and the histopathologic patterns found in skin biopsies positive for B19V DNA are discussed.

Materials and methods

Case selection

A total of 1,815 cutaneous biopsy specimens from 1,749 patients were selected for PCR detection of B19V. Criteria for selection were either 1) derived from the accompanying clinical information on the request form (suggesting a clinical diagnosis of erythema infectiosum, reticulate or other form of exanthema of possible viral origin, or PPGSS; 2) a histopathologic pattern suggestive of virus-related exanthema, particularly when a superficial perivascular lymphocytic infiltrate accompanied by extravasated red blood cells or an interstitial granulomatous dermatitis pattern (as described in 2000 by Magro et al[10]), were present, or 3) clinico-histopathologic diagnosis of some of the nosological entities or dermatologic pictures that have been related to B19V infection in the medical literature.

Clinical data and histopathology review

All available medical records, laboratory data and glass slides (haematoxylin and eosin in all cases, and special stains and immunohistochemical study for VP2 structural protein when performed) were reviewed by two of the authors (CS and LR). The clinical diagnoses (as suggested by the physicians submitting the cases on requisition slips) and the histopathologic patterns found in the biopsies were grouped and tabulated.

Immunohistochemistry

Among the PCR-positive cases, 92 cases were selected for immunohistochemical study of VP2 protein. These corresponded to 3 instances of PPGSS and 89 cases arbitrarily selected because of a histopathologic picture comprising perivascular mononuclear inflammation, red blood cell extravasation and mild epidermal changes, similar to the findings in PPGSS. Polyclonal Rabbit Anti-Parvovirus B19 VP2 protein (DakoCytomation, Denmark) was used,

and visualization was achieved with Dako EnVision system. Positive and negative controls were simultaneously studied.

Polymerase chain reaction

Detection of Parvovirus B19 was performed from DNA isolated from formalin-fixed, paraffin-embedded tissue.[27] Briefly, amplification of 50 ng DNA using specific primers for the B19V genome[17] was performed in a PTC 200 thermocycler (MJ Research, Inc. Watertown, Massachusetts, USA) with 50 pmol of each primer, 10 mM Tris-HCl, 50 mM KCl, 1,5 mM MgCl2, 200 mM of each dNTP, 1,5 U Taq-Polmerase. The initial annealing temperature was 65ºC decreasing to 55ºC for the final 35 cycles. Sequencing of the 422 bp PCR product was carried out with the dye terminator sequencing kit (Amersham Pharmacia Biotech, Freiburg, Germany) following the instructions of the suppliers. In every PCR run two mixes without DNA and two mixes with Parvovirus B19 DNA from formalin fixed, paraffin embedded tissue were used as negative and positive controls, respectively (with 8 samples at maximum). All PCRs, including the controls, were done in duplicate. All negative controls gave negative results.

Results

B19V DNA was detected in 402 out of 1,815 biopsy specimens (22%) from 394 patients. B19V VP2 structural protein was detected by immunohistochemical study only in the 3 instances of PPGSS (as reported elsewhere[40]), with involvement of endothelial cells of small blood vessels of the papillary dermis (Fig. 1). This consisted of granular positive material within the cytoplasm of endothelial cells; no staining was observed in the nuclei of the endothelial cells.

247 patients were female, 145 were male, and in 2 patients sex was not specified in the accompanying report and could not be assigned. Age range was 4 to 82 years of age (mean, 35.3; median 35; standard deviation 16.3). Site of biopsied lesions was the head in 10 cases (2.5%), trunk in 108 cases (26.8%), and extremities in 222 cases (55.1%); in 62 cases (15.4%) no information was given as to site of biopsy.

Histopathologic prevailing patterns of inflammation and their frequencies are detailed in Table 1. Although in these biopsies there was a clear predominance of perivascular inflammation, including variants with lichenoid pattern, ballooning or spongiosis of the epidermis, other histopathologic inflammatory patterns were also represented. The proposed clinical diagnoses in the requisition slips were tabulated. Since in many cases more than one diagnosis was suggested, the total number of entries amounts to 586. A total of 111 individual proposed clinical diagnoses were identified, covering a wide spectrum of dermatologic entities, which we have clustered in 11 groups (Table 2, supplementary material). Most frequent were the clinical diagnoses of vasculitis (40 instances), pityriasis lichenoides (38), pityriasis rosea (33), dermatitis/eczema (27), lichen planus/lichen nitidus (19), psoriasis (18), purpura (18), erythema chronicum migrans/borreliosis (17) and granuloma annulare (16). In 10 or more cases the proposed diagnoses were drug eruption, viral exanthema, lupus erythematosus, allergic vasculitis, lymphoma, parapsoriasis, sarcoidosis, urticaria, erythema multiforme and Sweet syndrome. No diagnosis was given or it was illegible in 29 instances. Final interpretation after histopathologic study was in many cases descriptive, with a comment regarding the most likely diagnosis in view of the submitted clinical information and proposed dermatologic diagnoses.

Discussion

Our study firmly establishes that around 20% of skin biopsies harbour B19V DNA, expands the spectrum of clinical and histopathologic dermatologic conditions and patterns in which B19V DNA may be found, and casts doubts on the alleged etiopathogenic significance of a positive B19V PCR in cutaneous specimens.

Eight years after its discovery in 1975, B19V was shown to be the cause of erythema infectiosum, a common childhood disease with a characteristic erythematous maculopapular rash (reticulated erythema) involving the trunk and the face of symptomatic patients, often resulting in a "slapped-cheek appearance", which is most likely caused by immune complex deposition.[5] In early studies, epidermal cells from a biopsy of the reticulated erythema in a child were shown to harbour viral proteins (by indirect immunofluorescence), and DNA (by in situ hybridization);[9] likewise, viral particles (by electron microscopy) and viral protein (by immunohistochemistry) were identified in the cytoplasm of vascular endothelial cells in an adult patient.[47] Other dermatologic conditions were later on associated with B19V infection in the light of serologic proof of recent infection and/or polymerase chain reaction (PCR) detection of B19V DNA (either in serum, tissue, or both). There is no doubt that acute B19V infection brings about either atypical exanthemas[48] or a variety of dermatologic patterns, recently grouped by Mage et al into exanthema (reticulated and annular), gloves-and-socks, periflexural and palpable purpura.[6] However, the assumption that detection of B19V DNA in a skin biopsy equals to pathogenicity seems no longer tenable, both from the available data in the literature in smaller series,[25,42,44,45,49] and from the present, more comprehensive study. Earlier studies disclosed B19V DNA in 40% of 36 cutaneous biopsies of patients with urticaria, and in 64% of 22 healthy volunteers.[25] In their 2006 paper proposing the concept of Bioportfolio, Norja et al studied, apart from the skin, samples from synovium, tonsil, liver and serum, and found 48% positivity in a series of 140 cutaneous biopsies from "B19-

unrelated dermatological lesions(…) and from healthy hospital staff".[42] 29 out of 38 skin biopsies for conditions unrelated to B19V were positive for viral DNA in the work of Corcioli et al.[49] Our 22% figure is close to that in the study by Bonvicini et al,[45] in which B19V DNA was detected by PCR and in situ hybridization techniques in 28% of pytiriasis lichenoides biopsies, 26% of melanocytic naevi, 17% of primary melanomas and 25% of normal skin specimens (total number of skin samples, 121). Higher values were found by nested and semiquantitative PCR by Sidoti et al: 40% of healthy skin controls, 41% of a variety of inflammatory dermatoses (psoriasis, atopic dermatitis, contact dermatitis, drug reactions, drug-versus host disease), and 34% of cutaneous T-cell lymphomas (total number of biopsies, 198). harboured B19V DNA.[44] The skin appears therefore to be but one of the many organs in which B19V can be detected both in healthy individuals and in patients with diverse dermatologic ailments.

Other than the skin, the list of healthy and diseased tissues allegedly harbouring B19V DNA or VP2 protein includes, in alphabetical order, bone marrow, brain, colon, heart, kidney, liver, lung, lymphoid tissue, small intestine, stomach, synovium, testis and thyroid.[50,51]This has led to claims of involvement of the virus in acute myocarditis and dilated cardiomyophaty,[52] Kikuchi-Fujimoto disease,[53] malignant lymphomas,[54] non-occlusive gangrene of stomach and bowel,[51] colon carcinoma,[55] testicular neoplasia,[56] and thyroid disorders.[57] The initial enthusiasm in the cardiology literature has somewhat subsided, in view of the evidence for lifelong persistence of B19V DNA in the myocardium.[58] The mechanism of viral persistence is unknown, but it is hypothesized (but by no means proven) that the virus might establish a latent-like type of infection, with harmless low DNA level resulting from inability to replicate in non-permissive cells;[50] however, the possibility of reactivation can not be ruled out[59]

The tropism of B19V for endothelium, due to endothelial cells also expressing the viral receptor P antigen[60] has been studied by several means. On the few reported immunohistochemical investigations of cutaneous biopsies for VP2, endothelial positivity has been so far restricted to the cytoplasm,[17,40,47] although involvement of endothelial cell nuclei has been elegantly demonstrated in placental chorionic villi.[61] The finding of B19V protein, DNA and messenger ribonucleic acid, supports a role for B19V in vascular lesions that can lead to full-blown pathologic syndromes, including myocardial infarction/myocarditis,[62] systemic sclerosis,[63] vasculitis and autoimmune disorders.[41,64] In the context of cardiomyopathy, a role for B19V infection of bone-marrow derived circulating angiogenic cells has been recently suggested; this would lead to dysfunctional vascular repair in the myocardium.[65] Of note, it has recently been proposed[66] that the predominant mechanism of infection of endothelium by B19V involves antibody-dependent enhancement, with interaction between the virus-antibody complexes and factor C1q and its receptor, CD93. This would be in keeping with the detection of VP2 in the endothelium in our PPGSS cases, but it remains to be seen whether it represents evidence of an abortive infection or a productive one leading to apoptosis or to an inflammatory immune response.[2]

In summary, there is a wide array of dermatologic lesions, both from a clinical and a histopathologic standpoint, in which B19V DNA can be detected by PCR. In the absence of other proofs of recent infection, this finding is not particularly supportive of any etiopathogenic role for the virus in this varied group of cutaneous ailments.

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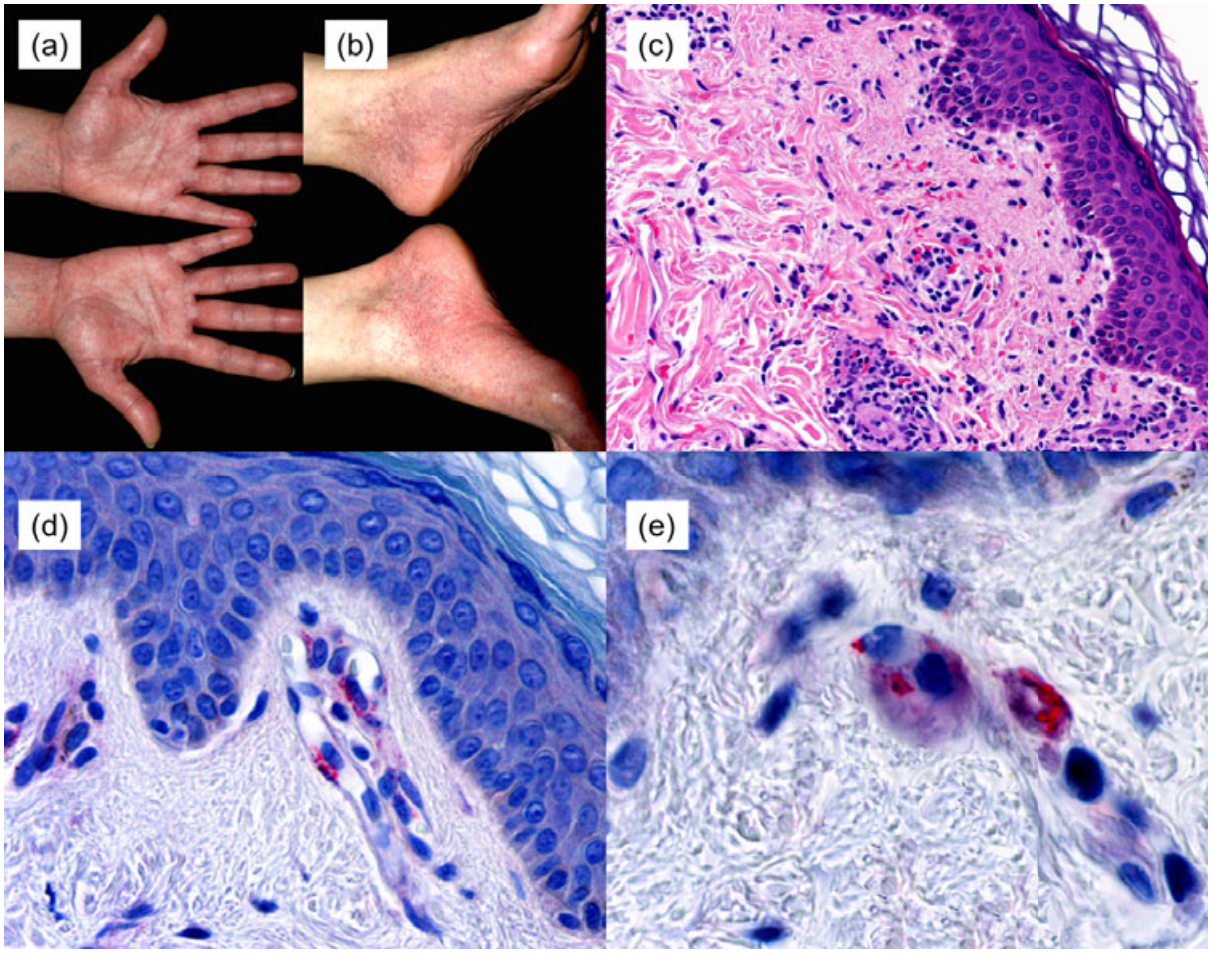
Table 1 Patterns of inflammation/histologic findings in 402 biopsy specimens in which B19V DNA was detected by PCR

Histologic Pattern	**Number of cases (%)**
Perivascular only	72 (18%)
Perivascular with lichenoid infiltrate	68 (17%)
Perivascular with cell ballooning	11 (2.7%)
Perivascular with spongiosis	79 (20%)
Perivascular with psoriasiform epidermal hyperplasia	10 (2.4%)
Nodular with predominance of lymphocytes	3 (0.7%)
Nodular with predominance of histiocytes	20 (5%)
Diffuse with predominance of lymphocytes	3 (0.7%)
Diffuse with predominance of neutrophils	11 (2.7%)
Diffuse with predominance of histiocytes	8 (2%)
Small vessel vasculitis	23 (5.7%)
Large vessel vasculitis	1 (0.2%)
Vaso-occlusive vasculitis	1 (0.2%)
Intraepidermal vesicular	2(0.5%)
Melanocytic Naevus	2 (0.5%)
Ulceration	1 (0.2%)
Normal skin	1 (0.2%)



Carlos Santonja, Úrsula Pielasinsky, Jorge Polo, Heinz Kutzner, Luis Requena

Immunohistochemical demonstration of Parvovirus B19 VP2 protein in periflexular exanthema in an adult, supporting antibody-dependent enhancement as means of endothelial uptake of the virus.

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RE: AJD-D-17-00161R1, titled "Immunohistochemical demonstration of Parvovirus B19 VP2 protein in periflexular exanthema in an adult, supporting antibody-dependent enhancement as means of endothelial uptake of the virus"

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Immunohistochemical demonstration of Parvovirus B19 VP2 protein in periflexular exanthema in an adult, supporting antibody-dependent enhancement as means of endothelial uptake of the virus

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Immunohistochemical demonstration of Parvovirus B19 VP2 protein in periflexular exanthema in an adult, supporting antibody-dependent enhancement as means of endothelial uptake of the virus.

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ABSTRACT

Human Parvovirus B19 (B19V) causes a number of skin exanthemas and has been related to both cutaneous and systemic diseases. Tropism of the virus for the rapidly proliferating erythroid progenitor cells in the bone marrow and fetal liver explains the pathogenesis of anemia and fetal hydrops. The cutaneous lesions of erythema infectiosum and other B19V-related exanthemas have been attributed to deposition of immune complexes in the skin. We report on the immunohistochemical detection of B19V protein in the cytoplasm of dermal endothelial cells in a case of periflexural exanthema in a 28-year-old woman. An antibody-dependent enhancement mechanism of entry has been suggested for B19V in myocardial endothelial cells, and could also be involved in B19V-related exanthemas.

Keywords

Parvovirus B19 Immunohistochemistry Antibody-dependent enhancement Skin Pathogenesis

INTRODUCTION

Primary infection by Human Parvovirus B19 (B19V), the etiological agent of erythema infectiosum in infancy and childhood, can result in cutaneous lesions in adults, with patterns designated as exanthema, “gloves and socks”, periflexural and palpable purpura [1] that can either appear isolated or overlap. Moreover, around 5% of atypical exanthemas (i.e. those whose morphology and etiology depart from the six classical ones) [2] have been found to be related to B19V infection, both in children and in adults. These skin manifestations are thought to be due to specific immunological reaction to the virus. In the rarely biopsied cases of primary B19V infection, a dermal mononuclear perivascular lymphocytic infiltrate with erythrocyte extravasation, as well as leukocytoclastic vasculitis, have been reported. Immunohistochemistry has surprisingly been used only anecdotally, but it can reveal viral proteins in epidermal keratinocytes or within endothelial cells of the superficial dermis, a finding of uncertain significance. [3,4] We report for the first time on the immunohistochemical detection of B19V VP2 protein within dermal endothelial cells in periflexular and purpuric involvement in primary B19V infection in an adult patient, review previous reports in the literature and discuss the possible relationship of this finding with the recently described antibody-mediated enhancement (ADE) as uptake mechanism of B19V. [5]

MATERIALS AND METHODS

The skin biopsy was fixed in formalin and embedded in paraffin. 5-micron sections were stained with hematoxylin&eosin. Immunohistochemical study of B19V viral protein 2 (VP2) structural protein (Polyclonal Rabbit Anti-PVB19, DakoCytomation, Denmark) was performed following the manufacturer´s instructions and was visualized with DAKO EnVision system. Liver tissue from a B19V-associated fetal hydrops was used as positive control. Polymerase chain reaction (PCR) techniques for molecular detection of B19V were performed according to Aractingi et al. [3] The serological detection of antibodies against B19V was done by enzyme-linked immunosorbent assay.

RESULTS

Clinical History

A 28-year-old caucasian woman was seen at the Emergency Department of Fundación Jiménez Díaz with a 24-hour history of fever, pruritus and a rash. She described the latter as macules and papules that had first appeared on her vulva, inguinal folds and thighs, spreading later on to her axillae, arms and legs. Her previous medical history included type II diabetes, obesity and a non-secreting pituitary microadenoma diagnosed several years earlier in Cuba (her home country), for which she received Linagliptin and Metformin. On admission, her temperature was 37.6ºC. She had mild tachycardia and normal blood pressure. On examination, an extensive exanthema of

erythematous papules with petechial lesions was observed involving particularly axillae, anogenital region, inner thighs, buttocks and antecubital fossae (Figure 1). She had no lesions in oral mucosa, palms or soles. Laboratory data showed leukocytosis (15,820 cells per microliter), with neutrophilia, mild hyperglycemia (serum glucose 127 mg/dl), and a mixed hyperbilirubinemia. Serum tryptase levels were normal. Urinalysis disclosed pyuria, hematuria and bacteriuria. Abdominal ultrasonography and CT-scan revealed cholelithiasis with no signs of cholecystitis, and left renal lithiasis without dilatation of the excretory system. With the clinical suspicion of urinary versus biliary tract infection the patient was admitted, receiving antibiotic coverage and antihistamines. Dermatological evaluation the following day suggested baboon/like syndrome caused by primary Parvovirus B19 infection. Clinical evolution was favorable, with disappearance of fever and gradual fading of her cutaneous lesions over the course of 7 days. During her hospital stay she developed pancytopenia, with nadir values of white cell count, hematocrit and platelets of 1,360 cells/µl, 29.6 %, and 102,000 cells /µl respectively. All these parameters spontaneously had returned to normal four weeks later. Serologic results for hepatitis A, B, and C, syphilis, HIV, Epstein-Barr virus, cytomegalovirus, rheumatoid factor, and antinuclear and antineutrophil cytoplasmic antibodies were negative. A serologic test for Parvovirus B19-specific IgM antibodies to viral capsid antigen was positive.

Pathologic Features

A skin biopsy performed on the 3rd day of admission showed unremarkable epidermis and a moderate, mostly perivascular mononuclear inflammatory infiltrate with some eosinophils and rare extravasated erythrocytes (Figure 2). No evidence of vasculitis was

seen. An immunohistochemical study for B19V VP2 protein revealed positive granular material within the cytoplasm of superficial dermal blood vessels (Figure 3).

DISCUSSION

Primary infection by B19V (family *Parvoviridae*, genus *Erytrhoparvovirus*, species *Primate erythroparvovirus 1*) is involved in two dermatological conditions with distinct cutaneous eruptions, namely erythema infectiosum in childhood and papular and purpuric "gloves and socks syndrome" (PPGSS) in adults. It can also cause other exanthemas in adults and children, designated palpable purpura, periflexural and atypical exanthemas. [1] [2]Serological conversion (i.e. IgM followed by IgG positive serology) and detection of viral deoxyribonucleic acid (DNA) in serum by PCR have linked these dermatologic manifestations to PVB19. The presence of B19V deoxyribonucleic acid (DNA) in biopsy specimens needs to be matched to serum samples, in order to confirm an acute infection, since the virus can persist for a long time in a number of tissues, including the skin. [6,7] Thus, the wide array of cutaneous ailments that along the years have been assumed to be related to PVB19 needs to be critically reviewed, accepting only those instances in which seroconversion and/or serum B19V can be proven (Table 1)
[3,8-37]

The specific tropism of PVB19 for bone marrow erythroid progenitor cells (due to their expression not only of the globoside receptor/P antigen but also of co-receptors Alpha5Beta1 integrin and Ku80), and the consequent cessation of erythropoiesis explain some of the pathological conditions with which PVB19 is associated: anemia in

immunocompromised patients, transient aplastic crises and hydrops fetalis.[38] Other ailments, such as arthropathy and the above mentioned cutaneous eruptions, have been attributed to viral activation of synoviocytes or to deposition of immune complexes, respectively. [39,40]

Few reports have addressed the cutaneous histopathologic findings of exanthemas caused by PVB19. The described lesions consist for the most part of extravasation of erythrocytes and a perivascular lymphohistiocytic infiltrate [3,4,41,42], but other patterns have been reported, among them vesicular, bullous or pustular lesions [43] [32,44], necrosis of keratinocytes [25] , interface dermatitis [27] , interstitial eosinophilic infiltrates [30] and cutaneous vasculitis [18,21,29,45] (Table)

Other than by means of PCR detection of B19V DNA, a specific search of B19V virion components in skin specimens has been performed occasionally. As early as 1994, Schwarz et al [46] described the presence of B19V viral particles by electron microscopy (EM) and indirect immunofluorescence within cells of the epidermis in a skin biopsy of the exanthema of erythema infectiosum in a child. One year later, Takahasi et al reported the involvement of vascular endothelial cells in an adult patient with primary B19V infection, demonstrating within their cytoplasm viral particles by EM and viral protein by immunohistochemistry. No immune complexes were identified by immunofluorescence, although C3 was deposited in the perivascular regions of the dermis. [47] A few years later, viral proteins were detected by immunohistochemistry in in three cases of papular-purpuric “gloves and socks” syndrome (PPGSS). The staining was seen not only in dermal endothelial cells, but also in keratinocytes and sweat gland epithelial cells. [3] In situ reverse transcriptase PCR (RT-PCR) was used by Magro et al [48] to demonstrate viral RNA in endothelial cells (and adjacent mononuclear cells) in skin biopsy specimens of several patients with positive IgG/IgM B19V serology and

clinical manifestations of connective tissue disease (including petechial and purpuric eruptions and confluent rashes). The same method was employed to demonstrate the virus in cases of endothelialitis allegedly secondary to B19V infection. [49] [50] Finally, positive immunohistochemical staining of the endothelium was reported in 2011 in three instances of PPGSS. [4]This study was negative in an additional case of mixed PPGSS and “bathing trunk” eruption.[37] In all cases in which the endothelium has been shown to contain B19V VP2 by immunohistochemistry, this was restricted to granular material within the cytoplasm of endothelial cells, even though nuclear localization of this protein should be expected and has been elegantly demonstrated in infected placental tissue with the same technique [51]

In the natural history of PB19V infection, an initial viremic phase (clinically undetected) allows the virus to gain access to permissive bone marrow erythroid progenitor cells, where they actively replicate and cause apoptosis, with release of progeny virus in the blood.[52] The normally nonpermissive endothelium has been recently shown to allow the entrance of PVB19 by means of ADE (in which B19V-antibody complexes use complement factor C1a and CD93 surface protein to facilitate endocytosis)[5]. Thus, the immune response to the virus in form of IgM antibodies contributes to its spread to other tissues, including the skin, synovial joints or endothelial cells of the myocardium. This infection, however, seems to be nonproductive, so that only under specific conditions (hypoxia, cytokine stimulation) would endothelial cells support the syntyhesis of viral proteins. [5]The detected VP2 in our case could represent unpackaged B19V particles, acquired by the ADE mechanism. It remains to be seen whether damage to the endothelium could follow, as has been speculated in the pathogenesis of B19V-related vasculitis and autoimmune diseases. [48,50,53]

In summary, we present the first case of B19V-related flexural-type exanthema with immunohistochemical demonstration of viral proteins within the cytoplasm of superficial dermal vessels endothelium, and propose that ADE-mediated uptake is responsible for this finding, as has been hypothesized for the endothelium of myocardial vessels.

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Figure legends

Figure 1. Papular and purpuric exanthema involving inguinal regions (a), antecubital fossa (b) and axillas (c,d) in a 28-year-old woman with fever and positive IgM for B19V.

Figure 2. Histopathologic appearance of a skin biopsy, showing mild perivascular, mostly lymphohistiocytic inflammation. No evidence of vasculitis was found.

Figure 3. Immunohistochemistry for B19V VP2. Note granular material within the cytoplasm of the endothelial cells of the upper dermis.

Figure 1

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Figure 2

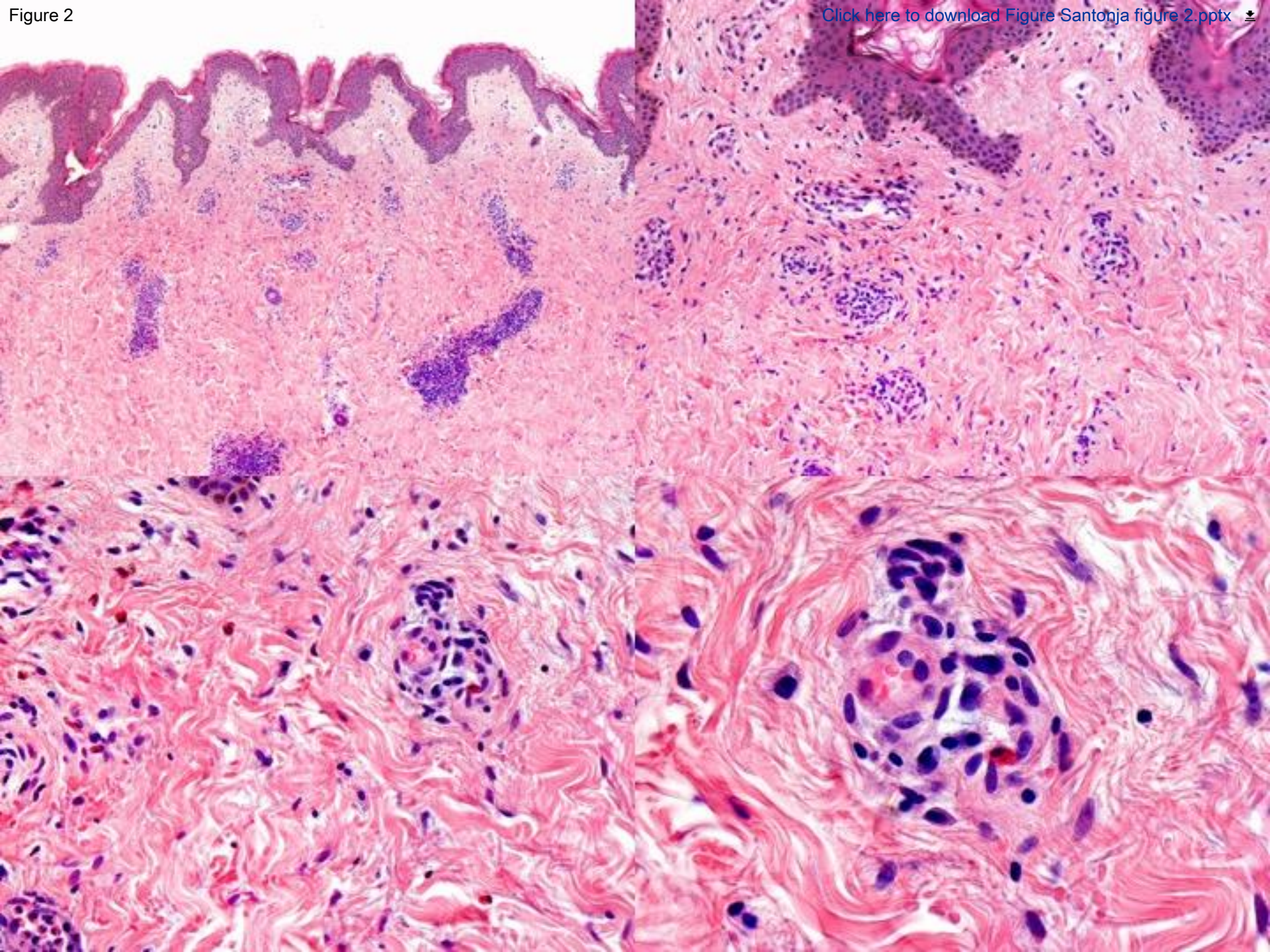

Figure 3

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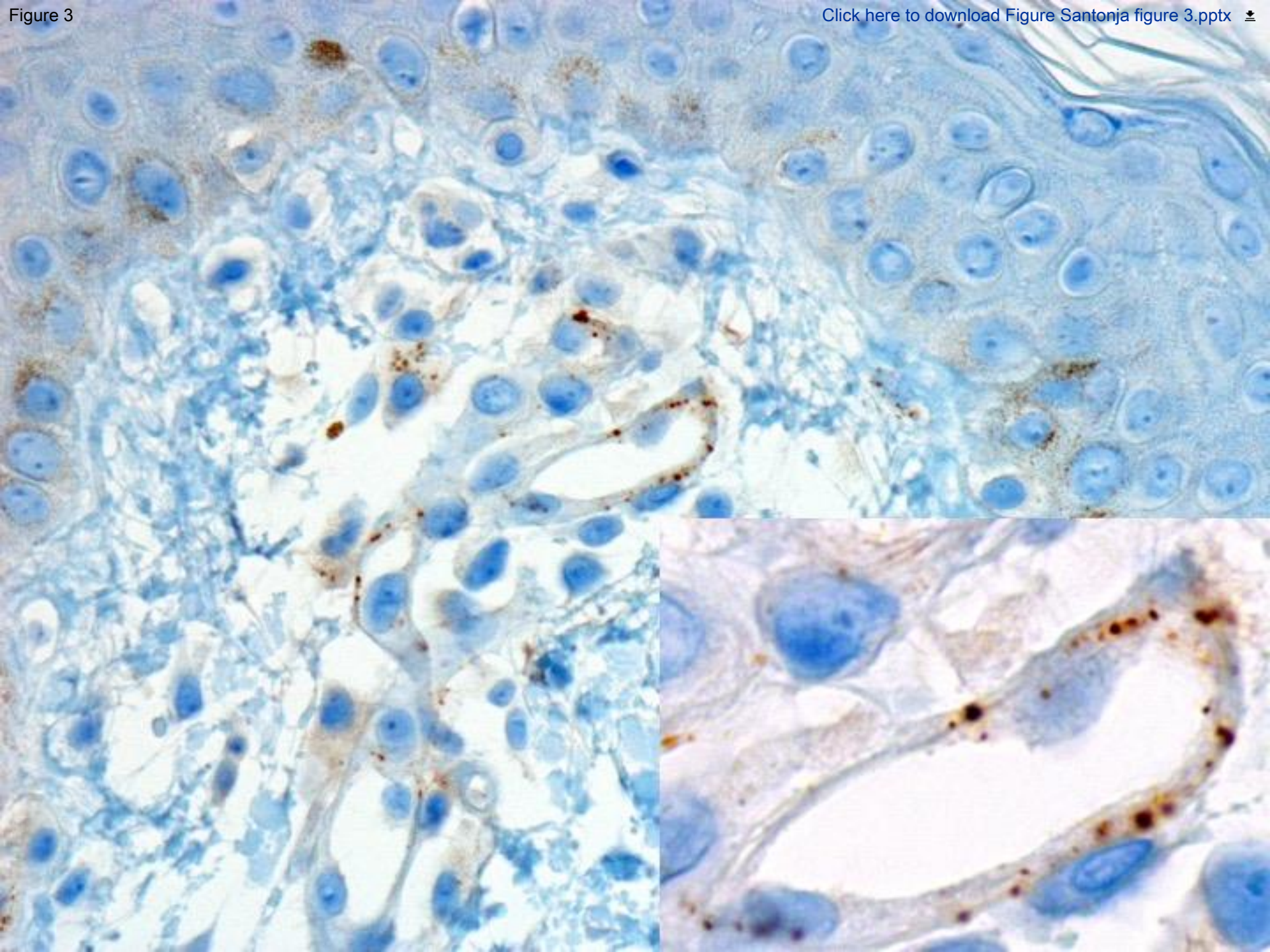

Revised Table

Table 1. Dermatologic clinical entities associated with B19V in the literature, pathologic findings in biopsies and means of B19V detection.

DISEASE/CLINICAL PATTERN	PATHOLOGIC FINDINGS IN SKIN	B19V Serum Antibodies	Serum B19V	Tissue B19V PCR	IHC detection of B19V
Erythema Infectiosum[9,46,10]	No biopsy[9] No details given[46] Interstitial/perivascular dermatitis[10]	IgM	ND	ND	ND
Henoch-Schönlein[11]	No biopsy	IgM	ND	ND	ND
Papular Petechial Vesiculopustular Eruption[43]	Vesiculopustules, Eosinophils	IgM, IgG	ND	ND	ND
Pruritic Macular Erythematous Rash[12]	No biopsy	IgM, IgG	ND	ND	ND

Pruritus, measles like rash[12]	No biopsy	IgM, IgG	ND	ND	ND
Pruritic rash[12]	No biopsy	IgM, IgG	ND	ND	ND
Erythema nodosum[13]	No biopsy	IgM	ND	ND	ND
Erythema Multiforme[14]	E. Multiforme	IgM, IgG	-	ND	ND
Keratolysis Exfoliativa[15]	No biopsy	IgM, IgG	ND	ND	ND
Papular purpuric “gloves and socks” syndrome[3,4]	Slight acanthosis, PVD, purpura	ND IgM, IgG	ND +	ND +	ND +
Morbilliform rash[16]	No biopsy	IgM	ND	ND	ND
Gianotti-Crosti[16]	No biopsy	IgM	ND	ND	ND
Purpura, Koplik spots[17]	No biopsy	IgM, IgG	ND	ND	ND
Palpable Purpura, Wegener Granulomatosis[18]	Leukocytoclastic Vasculitis	IgM, IgG	+	+	-

Livedo Reticularis and Myasthenia[19]	Vascular Telangiectasias	IgM	-	ND	ND
Polyarteritis Nodosa[18]	Leukocytoclastic Vasculitis	IgM, IgG	+	+	ND
Unilateral laterothoracic exanthem[20]	PVD + Hemorraghe	IgM, IgG	-	ND	ND
Papular Dermatitis Sweet-like Granuloma annulare–like Dermatomyositis–like Lupus-like Palpable Purpura[10]	Incipient GA: interstitial infiltration Interface Dermatitis Vasculitis, lymphocytic or granulomatous Dermal mucinosis Leucocytoclastic Vasculitis	IgM, IgG	ND	+	ND

Connective Tissue Disease (DM, LE, Vasculitis, PAN)[21]	Vacuolar Interface Dermatitis, incipient GA, mucinosis, PVD, Leucocytoclastic Vasculitis	IgM, IgG	-	+	ND
Digital Ischemia[22]	No biopsy	IgM, IgG	ND	ND	ND
Chronic urticaria Normal skin[23]	Not described	IgG	-	+	ND
Pseudo-cellulitic plaques[24]	No biopsy	IgM	ND	ND	ND
Pityriasis lichenoides[25]	Wedge-shaped infiltrate, extravasated erythocytes, variable necrosis of keratinocytes	ND	ND	+	ND

Baboon Syndrome-like[26]	PVD Hemorrhage Interface Dermatitis	IgM	+	ND	ND
Asymmetric Periflexural Exanthem[27]	Interface Dermatitis, Syringotropic lymphocytic infiltrate	IgM, IgG	ND	ND	ND
Psoriasis[28]	Not applicable	IgG, IgM	+	ND	ND
Behçet's Disease[29]	Leukocytoclastic vasculitis	IgG	ND	+	ND
Wells' syndrome[30]	Eosinophilic interstitial infiltrate with flame figures	IgM, IgG	+ (ND)	+(ND)	ND
Melkersson-Rosenthal syndrome[31]	Multiple granulomas in lip and paranasal sinus	IgG	+	ND	ND

Hydroa vacciniforme[32]	Intraepidermal bullae, lymphocytic dermal infiltrate	ND	ND	+	ND
Sweet's syndrome[33]	Dermal neutrophils, also lymphocytes and plasma cells	IgM, IgGH	ND	ND	ND
Febrile ulceronecrotic Mucha-Habermann disease[34]	Psoriasiform dermatitis, exocitosis of lymphoid cells, dermal edema and erythrocyte extravasation	IgM, IgG	+	-	ND

Acute Exanthematic Pustulosis[35]	Subcorneal pustules, dermal perivascular and interstitial inflammation	IgM, IgG	ND	ND	ND
Flagellate Erythema[36]	No biopsy	IgM	ND	ND	ND
Papular purpuric “gloves and socks” syndrome and bathing-trunk eruption[37]	Lymphocytic exocytosis, edema in dermis, extravasated red blood cells, perivascular lymphohistiocytic infiltrate	IgM, IgG	ND	ND	-

Present Case Periflexural exanthema	Perivascular mononuclear inflammatory infiltrate, eosinophils, extravasated erythrocytes	IgM	ND	ND	+

PCR, Polymerase Chain Reaction; IHC, immunohistochemical; PVD, Perivascular Dermatitis; +, Positive; -, Negative; ND, Not Done